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ERDEC-TR-372

DERMAL TOXICITY EVALUATION OF NEUTRALIZED CHEMICAL AGENT IDENTIFICATION SETS (CAIS) WITH AN OVERVIEW OF THE DERMAL TOXICITY OF VESICANT AGENTS AND THEIR DEGRADATION PRODUCTS

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RESEARCH AND TECHNOLOGY DIRECTORATE

October 1996

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AUTHORS	Eugene J. Olajos, Kenneth P. Cameron, Ruth A. Way, James H. Manthei, Dale H. Heitkamp, Dean M. Bona, and Sandra A. Thomson
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13. ABSTRACT *(Maximum 200 words)*
Acute dermal toxicity (limit test) and skin irritation studies were conducted in New Zealand white rabbits to ascertain the systemic toxicity and skin-injurant potential of chemically-neutralized Chemical Agent Identification Sets (CAIS). Studies also included the assessment of oxidant/solvent systems and solvent induced toxicity. The toxicity limit test consisted of a 24-hr occluded exposure to 1.0 ml/kg of "test article." Dermal irritation studies were based on a 4-hr occluded exposure to 0.5 ml of "test article." Chemical neutralization of CAIS resulted in complex product solutions (wastestreams) containing ppm levels of agent and an array of degradation products. Findings from the skin irritation testing of wastestreams and oxidant/solvent systems indicate that wastestream-induced skin effects (edema and erythema) were equivalent to or less in severity than the skin effects produced by exposure to oxidant/solvent systems. Systemic effects were not observed in 4/5 wastestream-exposed groups; however, 2/5 wastestream-treated groups exhibited systemic effects. Lethality was noted in only 1/5 wastestream-treated groups. Limit test data indicate that agents (HD, HN, or L) were destroyed by reaction with oxidant to less toxic materials.

14. SUBJECT TERMS

Wastestreams	DOT	L	Systemic toxicity
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Chemical agents	Rabbits	HN	Chemical Agent Identification Sets

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QUALITY ASSURANCE

This study, conducted as described by Protocol 210940440000 and associated annexes, was examined for compliance with Good Laboratory Practices as published by the U. S. Environmental Protection Agency in 40 CFR Part 792 (effective 17 Aug 1989). The dates of all inspections and the dates the results of those inspections were reported to the Study Director and management were as follows:

Phase inspected	Date	Date reported
Dosing, toxic signs	29 Mar 95	30 Mar 95
Data & Final Report	23 Sep 96	23 Sep 96

To the best of my knowledge, the methods described were the methods followed during the study. The report was determined to be an accurate reflection of the raw data obtained.

Dennis W. Johnson

DENNIS W. JOHNSON
QA Coordinator, Research & Technology

PREFACE

The work described in this report was authorized by Project Manager Non-Stockpile Chemical Materiel. This work was started in January 1995 and completed in September 1995. The experimental data are recorded in laboratory notebook nos. 87-0120 and 92-0015.

In conducting the research described in this report, the investigators adhered to the "Guide for the Care and Use of Laboratory Animals," National Institute of Health Publication 85-23, 1985, as promulgated by the Committee on Revision of the Guide for Laboratory Animal Facilities and Care of the Institute of Laboratory Animal Resources, Commission of Life Sciences, National Research Council (Washington, DC). These investigations were also performed in accordance with the requirements of AR 70-18, "Laboratory Animals, Procurement, Transportation, Use, Care, and Public Affairs," and the Laboratory Animal Use and Review Committee (LAURC), U.S. Army Edgewood Research, Development and Engineering Center (ERDEC), which oversees the use of laboratory animals by reviewing for approval all ERDEC research protocols requiring laboratory animals. This project, assigned LAURC Protocol No. 210944000, was approved on 30 Jun 94.

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Dermal Toxicity Evaluation of Neutralized Chemical Agent Identification Sets (CAIS) with an Overview of the Dermal Toxicity of Vesicant Agents and their Degradation Products

1. INTRODUCTION

The U.S. Army Chemical Materiel Destruction Agency (USACMDA) was established to demilitarize and dispose of obsolete chemical devices. The Project Manager for Non-stockpile Chemical Materiel (PMNSCM) is responsible for the demilitarization/ destruction of all non-stockpile chemical materiel(NSCM)- among those items are Chemical Agent Identification Sets (CAIS). CAIS are training items that contain small to fairly substantial quantities (i.e., HN content up to 10%) of chemical warfare agents [sulfur mustard (H, HS, or HD), nitrogen mustard (HN-1 or HN-3), or lewisite (L)]. CAIS may also contain miscellaneous materiel/industrial chemicals such as cyanogen chloride (CK), phosgene (CG), chloroacetophenone (CN), adamsite (DM) and chloropicrin (PS) as indicated in the CAIS information package (PMCD, 1995). CAIS that contain industrial chemicals are intended for recontainerization in accordance with DOT requirements and sent to a commercial hazardous waste disposal facility. CAIS that contain chemical agent (HD, HN, or L) will be chemically treated on site [Rapid Response System (RRS)][1] to products/residues having reduced toxicity/adverse health effects potential. The CAIS wastestreams (products/residues of agent deactivation) will be handled, transported, and disposed of in a manner similar to industrial wastes in accordance with regulatory requirements.

CAIS are classified by both variety and type [e.g., Toxic Gas Sets (DODAC Code K941, K942) containing neat sulfur mustard (H, HS, or HD); gas identification (detonation) sets containing sulfur mustard (5%) in chloroform, nitrogen mustard (HN-1) (10%) in chloroform, or lewisite (5%) in chloroform; and "Sniff Set" containing sulfur mustard, nitrogen mustard (HN-1 or HN-3), or lewisite on charcoal]. These sets were developed and manufactured by the U.S. Army and distributed to all branches of the armed services between 1928 and 1969. The purpose of these sets was to familiarize troops with the properties of CW agents (appearance, color, odor), and in the case of gas identification set (detonation) to include recognition of biological effects [e.g. irritation (eye, nose, throat) and prickling sensation (skin)-referred to in the training manuals as "immediate effects"]. CAIS were declared obsolete in 1971.

Previous R&D efforts (process chemistry development and toxicity testing) related to the demilitarization/detoxification

[1] **RRS transportable system for identification, segregation, repackaging and/or treatment of CAIS.**

of obsolete War Gas Identification/Toxic Gas Sets were conducted between 1973 and 1975 and the findings reported (Rescigno and Duggan, 1977; Rosenberg 1977). Although the toxicity studies cited focused on monoethanolamine (MEA)-neutralized HD, toxicologic investigations were not limited to mustard/MEA preparations - testing was also conducted on residuals from the reaction of MEA with L, CK, CG, or PS. These consisted of acute oral and dermal toxicity studies and skin/eye irritation studies of decontamination "residues" as well as i.v. screens on decontamination solutions, residues, and agent simulants. The intent was to develop a neutralization process chemistry that would produce a degraded CAIS that was "...... at least safe enough for shipment by DOT criteria" (Army memo, November 1972).

Recently-conducted and on-going chemistry and toxicology studies related to RRS stem not only from the need to develop effective chemical neutralization processes, (as was the case in the early 1970s involving the deactivation/detoxification of CAIS), but from the need for demonstrating marked reduction in agent characteristics. Thus, chemical neutralization processes were sought which (1) achieved process simplicity, (2) resulted in marked reduction in agent characteristics (i.e. vesication), and (3) which generated wastestreams having reduced toxicity (i.e. systemic toxicity) that can be handled and disposed of in a manner similar to that of industrial chemicals and/or wastes. Toxicity studies to ascertain the effectiveness of a process chemistry in degrading/detoxifying CAIS with minimal toxicity/adverse health effects potential included the following: acute dermal, dermal irritation, vesication, and inhalation toxicity tests. This report provides an account of the authors' investigations on the dermal toxicity potential of the various wastestreams resultant from the treatment of CAIS by various process chemistry technologies. Inhalation and vesication studies on CAIS wastestreams are on-going and the findings from these tests will be reported separately.

2. MATERIALS AND METHODS

2.1 Chemicals.

2.1.1 Test Materials.

2.1.1.1 Chemical Agents.

The chemical agents found in CAIS (neat, in chloroform, or on charcoal) include sulfur mustard (H, HD or HS), nitrogen mustard (HN-1 or HN-3), or lewisite (L). Sulfur mustard[2,2-dichloroethyl sulfide (HD), CAS #505-60-2] CASARM grade (97.5 mole %) was procured from Operations Directorate, ERDEC, APG, MD. Nitrogen mustard [bis (2-chloroethyl) ethylamine(HN-1) [purity ≥ 97%], CAS #538-07-8]; tris (2-chloroethyl)amine (HN-3)

[purity $\geq$ 97 %], CAS #555-77-1] and lewisite [dichloro-2-chlorovinyl arsine (L),CAS #541-25-3 CASARM grade (97.8% by weight] were procured from Operations Directorate, ERDEC, APG, MD. Physicochemical properties of these agents as well as the toxicologic properties are summarized in Appendices A and E.

2.1.1.2 Neutralized Chemical Agent Identification Sets (Wastestreams).

A total of five wastestreams (refer to Table 1 for process chemistry designation) from the chemical neutralization of CAIS **[Actual ampoules from CAIS kits were not used. Instead, "CAIS" were prepared from agent stocks to the following specifications (10% HD, HN, or L (Chatfield etal, 1995)).]** were tested for dermal toxicity. These wastestreams were as follows:

- **Wastestream from neutralization of neat sulfur mustard via 1,3-dibromo-5,5 dimethylhydantoin (Brom-55p)/(sulfolane/3% water).**
- **Wastestream from neutralization of neat sulfur mustard via 1,3-dichloro-5,5 dimethylhydantoin (DCDMH) in $CHCl_3$/t-BuOH/3% H_2O.**
- **Wastestream from neutralization of HD, HN-1, and L (agent in chloroform) via m-chloroperoxybenzoic acid (m-cpba) in $CHCl_3$/t-BuOH.**
- **Wastestream from neutralization of HD, HN-1, and L (agent in chloroform) via DCDMH in $CHCl_3$/t-BuOH/ 3% H_2O.**
- **Wastestream from neutralization of HD, HN-1, and L (agent on charcoal) via DCDMH in $CHCl_3$ (HD, HN-1) and via DCDMH in $CHCl_3$/t-BuOH/3% H_2O (L).**

2.1.1.3 Treatment Reagents (Oxidizers).

The following treatment reagents were procured: 1,3-dichloro-5,5 dimethylhydantoin (DCDMH) (CAS #118-52-5), and 1,3-dibromo-5,5 dimethylhydantoin (Brom-55p) (CAS #77-48-5), Aldrich Chemical Corp, St. Louis, MO; and m-chloroperoxybenzoic acid (m-cpba) (CAS#937-14-4), ICN Inc., Aurora, OH. Physicochemical properties as well as toxicological properties of these compounds are outlined in Appendices C and E.

2.1.1.4 Solvents

Process chemistries utilized one or more of the

following solvents: chloroform (CAS #67-66-3), t-butyl alcohol (CAS #75-65-0), and sulfolane (1,1-dioxothiolan) (CAS #126-33-0). Chloroform (optima grade, 99.9% purity) was procured from Fisher Scientific, Fairlawn, NJ. Tertiary-butyl alcohol (>99.7%) was obtained from Fluka Chemical Corp, Ronkonkoma, NY). Sulfolane was procured from Aldrich Chemical Co., St. Louis, MO. Physicochemical properties and toxicity characteristics for these compounds are outlined in Appendices D and E.

Table 1. Process Chemistries for Chemical Treatment (Neutralization/ Detoxification) of Chemical Agent Identification Sets (CAIS)

CAIS	Process Name	Oxidant (Treatment Reagent)	Solvent System
HD (neat)[a] (e.g. K941, K942 sets)	Blue (initial)	1,3-dibromo-5,5 dimethylhydantoin (Brom-55p)	Sulfolane/3% water[e]
HD (neat)	Blue (modified)	1,3-dichloro-5,5 dimethylhydantoin (DCDMH)[d]	Chloroform/t-butanol/ 3% water
HD,HN-1[b],L[c] in $CHCl_3$ (e.g. K951, K954 sets)	Red (initial)	m-chloroperoxybenzoic acid (m-cpba)	Chloroform/t-butanol
HD,HN-1,L in $CHCl_3$	Red (modified)	1,3-dichloro-5,5 dimethylhydantoin (DCDMH)	Chloroform/t-butanol/ 3% water
HD,HN-1,L charcoal	Charcoal (initial)	1,3-dichloro-5,5 dimethylhydantoin (DCDMH)	Chloroform
HD,HN-1,L	Charcoal (modified)	1,3-dichloro-5,5 dimethylhydantoin	Chloroform (HD,HN reactions) Chloroform/t-butanol (L reaction)

[a] HD (bis-(2-chloroethyl) sulfide); sets may have either H or HS in place of HD.
[b] HN-1 (bis-(2-chloroethyl) ethyl amine); predominant form of nitrogen mustard in CAIS containing charcoal; small proportion of charcoal sets contain HN-3.
[c] L (dichloro-(2-chlorovinyl) arsine)
[d] DCDMH (RH-195) has been previously used for HS decontamination.
[e] Sulfolane (anhydrous sulfolane containing 3% H_2O)

2.1.1.5 <u>Oxidizer/Solvent Systems</u>.

The following oxidizer/solvent system solutions (also

refer to Table 1) were tested for dermal toxicity:

Blue Process Reagents

- **1,3-dibromo-5,5 dimethylhydantoin/sulfolane/3% H_2O.**
- **1,3-dichloro-5,5 dimethylhydantoin (0.555M) in 50:50 $CHCl_3$/t-butyl alcohol with 3% H_2O.**

Red Process Reagents

- **m-chloroperoxybenzoic acid/$CHCl_3$/t-butyl alcohol.**
- **1,3-dichloro-5,5 dimethylhydantoin (0.555M)in 50:50 $CHCl_3$/t-butyl alcohol with 3% H_2O.**

Charcoal Process Reagents

- **1,3-dichloro-5,5 dimethylhydantoin (0.555M) in $CHCl_3$/t-butyl alcohol with 3% H_2O.**

2.2 Process Chemistries (Chemical Neutralization Technologies).

Various chemical neutralization processes have been developed for the chemical detoxification[2] of CAIS related to RRS. Process chemistries were driven by the need for effective chemical neutralization of agent as well as processes that are associated with minimal health risks. The neutralization processes vary with respect to: (1) oxidizer[3] [m-chloroperoxybenzoic acid (m-cpba), 1,3-dibromo-5,5 dimethylhydantoin (Brom-55p) or 1,3 dichloro-5,5 dimethylhydantoin (DCDMH)] and (2) solvent system [sulfolane/water; chloroform/t-butyl alcohol; chloroform/t-butyl alcohol/water]. The choice of treatment is based on CAIS composition [neat sulfur mustard (H, HD, or HS); agent (HD, HN-1 or HN-3, L) in chloroform; and agent (HD, HN-1, L) absorbed on charcoal]. A total of six process chemistries have been developed (initial and modified) and are outlined in Table 1. Formulations of treatment reagent/solvent systems (modified process chemistries only) for the conversion of

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[2] **Chemical detoxification is defined as a process to convert chemical agents to products that do not exhibit highly toxic properties of the chemical warfare material (CWM). This process is also known as chemical neutralization as defined in Army Regulation 385-61.**

[3] **DCDMH and Brom-55p are oxidants that selectively oxidize HD to the sulfoxide compared to those that further oxidize HD to the sulfone. DCDMH (RH-195) has been previously utilized by the military for HS decontamination (Brown, 1938).**

chemical agent (neutralization of CAIS) are highlighted in Table 2. The physicochemical properties of the treatment reagents, solvents, agents, and agent by-products (e.g., HD sulfone, HD sulfoxide) are summarized in Appendix E. Toxicologic profiles for the agents; key agent degradation products of HD, HN, or L; treatment reagents, and solvents are summarized in Appendices A - D. Using the neutralization processes, the chemical agent in the CAIS may undergo oxidation/chlorination/substitution to yield a mixture of products and by-products. Residual agent (HD, HN, or L) at the ppm level may also be present in the wastestream. Specifics of the process chemistries involved have been described via informal updates/reports and/or personal communications.

Table 2. Oxidizer/Solvent System Formulations Utilized for the Modified Blue, Red, and Charcoal Process Chemistries

- 1 volume of neat HD treated with 20 volumes of 0.555M 1,3-dichloro-5,5-dimethylhydantoin (DCDMH) in $CHCl_3$/t-butanol (50/50) with 3% water by volume.
- 1 volume of each 5-10% HD in $CHCl_3$, 5-10% HN1 in $CHCl_3$, and 5-10% L in $CHCl_3$ treated with 4 volumes of 0.555M 1,3-dichloro-5,5-dimethylhydantoin (DCDMH) in 50/50 $CHCl_3$/t-butanol with 3% water by volume.
- 43% by weight HD and HN-1 on charcoal treated with excess 1,3-dichloro-5,5-dimethylhydantoin in $CHCl_3$ combined with 43% by weight L with excess 1,3-dichloro-5,5-dimethylhydantoin in $CHCl_3$/ t-BuOH (50/50).

2.3 Analytical Methodology.

2.3.1 Agent Residue Analysis of Neutralized (CAIS)

Chemically-treated (neutralized) CAIS were analyzed for agent residue levels using full scanning GC/MS spectroscopy (for instrumentation and conditions refer to Table 3). The mass spectrometer utilized in the majority of analyses, via the electron ionization (EI) mode, was a Hewlett-Packard 5989B MS engine with Chemstation Data System. Electron energy was 70 electron volts (eV) and the emission current was 750 micro amps(uA). The instrument was equipped with a 30m x 0.25mm DB-5 capillary column using helium as carrier gas. Scan time was one second per scan and the range was 45 to 450 atomic mass units (amu). Quantitation was based on internal standardization (internal standard = 1,2,4,5 -tetrachlorobenzene). Calibration standards were as follows: HD (purity 97.5%) 36.7 ppm standard

in hexane; HN-1 (purity 96.5%) 55 ppm standard in chloroform; and L (purity 97.8%) 242.8 ppm standard in hexane. For procedural details, the reader is referred to Appendix F - "Method for the Determination of Chemical Warfare (CW) Agents in Neutralization Mixtures Using a Gas Chromatograph/Mass Spectrometer (GC/MS)".

Table 3. Instrumentation (GC-MS) Utilized in the Analyses of Wastestream Samples

Wastestream	Instrumentation	Instrument Parameters
I. Blue (Modified) [HD/DCDMH]		
Agent	EI[a]: Hewlett Packard MS Engine (5989B)	30m x 0.25 mm DB-5 capillary column[c], column flow 1.02 ml/min helium as carrier gas; Injection port temp 250°C; Temp programmed from 60-270°C 10°C/min)
Products	CI[b]: Finnigan 5100	15m x 0.25 mm RTX-5 capillary column[d]; GC/MS interface temp 230°C; Injection port temp 200°C; methane as CI reagent gas
Blue (Initial) [HD/ Brom-55P/sulfolane/ 3% H_2O]		
Agent	EI: Perkin-Elmer ITS40 ITD	Same as above EI parameters
Products	CI: Finnigan 5100	Same as above CI parameters
II. Red (Modified) [HD/HN/L DCDMH 3% H_2O 50/50 $CHCl_3$/t-BuOH]		
Agent	EI: Hewlett Packard MS Engine	Same as above EI parameters
Products	CI: Finnigan 5100	Same as above CI parameters
Red (Initial) [HD/HN/L/ m-cpba]		
Agent	EI: Hewlett Packard MS Engine	Same as above EI parameters
Products	CI: Finnigan 5100	Same as above CI parameters
III. Charcoal (Modified) HD/HN/L/ DCDMH $CHCl_3$/t-BuOH (L) $CHCl_3$/(HD,HN)		
Agent	EI: Hewlett Packard MS Engine	Same as above EI parameters
Products	CI: Finnigan 5100	Same as above CI parameters
Charcoal (Initial) [HD/HN/L DCDMH/$CHCl_3$]		
Agent	EI: Hewlett Packard MS Engine	Same as above EI parameters
Products	CI: Finnigan 5100	Same as above CI parameters

[a] EI: Electron ionization, full scan; 1,2,4,5-tetrachlorobenzene (internal standard).
[b] CI: Chemical ionization.
[c] DB-5 (95% Dimethyl-5%-diphenylpolysiloxane).
[d] RTX-5 (identical to DB-5).
[e] Other instrumentation utilized (Finnigan 5100, Perkin-Elmer)

2.3.2 Product Characterization of Neutralized CAIS (RRS Neutralization Wastestreams).

Product identification of the neutralized CAIS wastestreams was accomplished using GC/MS spectroscopy (EI and CI modes)[4] per procedures outlined in Appendix F. The predominant instrument for component identification via the chemical ionization (CI) mode was a Finnigan 5100 GC/MS. The mass spectrometer (Finnigan 5100) was operated in the chemical ionization (CI) mode with methane as the CI reagent gas at a source pressure of 0.5 Torr (for instrument conditions refer to Table 3). Scan time was one sec per scan, and the scan range was 60 to 450 amu.

2.4 Animals

2.4.1 Care and Treatment of Animals.

Young adult male and female New Zealand White (NZW) rabbits were procured by Veterinary Services, ERDEC from Charles River Laboratories, Wilmington, MA. On receipt animals were checked for general condition and health status and housed in Bldg E3222. The animal holding rooms were maintained at 75°F +/- 5 with relative humidity between 40-60%. Daylight/dark hours were automatically controlled on a 12-hr cycle. Food (Zeigler Certified Rabbit Chow) was provided ad libitum, as was water. Rabbits were housed individually in 8-unit stainless steel cages. Metal ear tags were used for positive identification during the test period. The animals were kept in quarantine for 7 days prior to testing. The nature of test material (decontaminated CW agent) required that all animals be held under restraint for a 24-hr period in accordance with Safety and Surety Standards Operating Procedures (SOP #CR-8-OSP18-95G). Since rabbits are especially susceptible to stress of prolonged immobilization, provisions were made for technique control which consisted of conditioning for restraint. Conditioning was conducted during the quarantine period. The animals were dosed with test material (wastestream, agent, oxidizer/solvent system, or solvent) as indicated in Table 4 and evaluated for dermal toxicity (systemic effects and irritation).

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[4] **CI spectra furnish molecular weight information to supplement EI spectral information providing positive identification. CI analysis is as routine as EI.**

Table 4. Animal Assignment and Study Phases

Study Phase	Number of Animals
Preliminary Screen/Limit Test (dermal 24-hr Contact)[a]	
Blue Process Chemistry (initial)	
- wastestream: HD/Brom-55p/sulfolane	2
- oxidizer/solvent: Brom-55p/sulfolane	8
Blue Process Chemistry (modified)	
- wastestream HD/DCDMH/t-BuOH/H_2O	10
- oxidizer/solvent: DCDMH/t-BuOH/H_2O	10
Red Process Chemistry (initial)	
- wastestream: (HD/HN/L)/m-cpba/$CHCl_3$/t-BuOH	4
- oxidizer/solvent: m-cpba/$CHCl_3$/t-BuOH	14
Red Process Chemistry (modified)	
- wastestream: (HD/HN/L)/DCDMH/$CHCl_3$/t-BuOH	20
- oxidizer/solvent: DCDMH/$CHCl_3$/t-BuOH	10
Charcoal Process Chemistry	
- wastestream: (HD/HN/L)/DCDMH/$CHCl_3$/t-BuOH	10
- oxidizer/solvents: DCDMH/$CHCl_3$/t-BuOH	(-)[b]
Dermal Irritation - 4-Hr (Components/Wastestreams)	
Blue Process Chemistry	
- initial	22
- modified	6
Red Process Chemistry	
- initial	22
- modified	54
Charcoal Process	
- modified	12
HN1 vs HN3	12

[a] **Dermal irritation also evaluated in these animals.**
[b] **Oxidizer/solvent system - same as for charcoal chemistry process.**

2.4.2 Toxicity Testing.

Dermal Toxicity Test Procedures (DOT):

Dermal toxicity assessment (LD_{50} and primary dermal irritation) of CAIS wastestreams and oxidizer/solvent systems was based on DOT guidelines [5] (CFR, 1993).

[5] **A primary consideration in determination of a toxicity test procedure is the specific requirements of regulatory agencies. Regulatory requirements frequently are the driving force in test methodology selection. The current DOT guidelines on hazardous materials transportation has its etiology in the early 1990s and was based on: (1) a DOT need for more rigorous toxicity database (i.e. LD_{50} test versus limit test) and (2) the assignment of hazardous materials to packing groups as opposed to "Class A" or "Class B" poisons as was the case under the pre-1990 DOT regulations. Further, DOT does permit classification based on toxicity data derived from testing conducted in accordance with other regulatory guidelines (i.e. U.S. EPA).**

The U.S. DOT (CFR, 1990) has promulgated criteria for acute toxicity testing (oral, dermal, inhalation) that permit classification of chemicals by degree of toxicity. Depending on classification, containerization, labels, etc, the intent is to prevent undue risk/injury in the transportation of industrial chemicals and goods.

2.4.2.1 Limit Test/Lethality Screen.

A single limit test and/or range-finding study, the goal of which is to quickly identify lethal and non-lethal doses, should precede an LD_{50} determination. Although acute toxicity data exist on the constituent components of the oxidant/solvent systems used in the various process chemistries, toxicity data are non-existent on mixtures containing these materials. Furthermore, limit test/lethality screens of oxidant/solvent systems and wastestreams were warranted for the following reasons: (1) Acute toxicity testing would define the relative toxicities of wastestreams, and more importantly that the chemical neutralization was effective in reducing the systemic toxicity of CAIS containing highly toxic materials [HD: dermal LD_{50} ($\approx$ 40 mg/kg); HN: dermal LD_{50} ($\approx$ 15 mg/kg); and L: dermal LD_{50} ($\approx$ 5 mg/kg)]; (2) Since degradation products from the chemical neutralization of CAIS are not necessarily "non-toxic" (HD sulfone, HN oxide have appreciable toxicity; divinyl sulfone, and 2-chlorovinylarsonous acid are highly toxic), toxicity testing would demonstrate whether the degradation products in the wastestreams pose an appreciable acute toxicity hazard; and (3) Since the toxicity assessment involves highly complex mixtures, one or more interactive effects (e.g., additive, synergistic, potentiation, antagonism) may contribute to an altered toxicity/spectrum of effects not seen on dosing with individual test substance. A limit test[6] was conducted on the oxidizer/solvent system and neutralized CAIS at a pre-determined level (1.0 ml/kg)[7]. Young adult male and female New Zealand White rabbits (2-3 kg, 3-5 months of age) were used. With the exception of the animals dosed with oxidizer/solvent and neutralized CAIS from the initial Blue Process (Brom-55p/ sulfolane), all treatment groups utilized a minimum of four animals. Test materials were applied to the clipped dorsal

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[6] **Historically (pre-1990), DOT had based toxicity classification of chemicals on limit tests. Current DOT regulations call for LD_{50} assessment but do not specify a limit test, as part of the LD_{50} test paradigm, as do other regulatory groups (EPA, OECD). Estimates of toxicity (i.e. dermal) of mixtures can be obtained only via animal testing (DOT, 49 CFR Part 107). When considering inhalation exposure, DOT permits the numerical estimation of the LC50 of a mixture from a formula where LC50 data is available on each of the components comprising the mixture. However, for highly complex mixtures, the likelihood that LC50 values exist for all constituents decreases, thus necessitating animal tests (i.e. Limit and/or LC50) (DOT, 49 CFR Part 107).**

[7] **Dose selection (1 g/kg = 1 ml/kg) is based on the following: (1) DOT considers a material as poisonous if the material has a dermal LD_{50} less than 1000 mg/kg (upper limit of 1000 mg/kg as poisonous) and (2) that 1 g/kg (1000 mg/kg) is a quantity of test article that can be reasonably applied topically.**

surface. The dose site was covered by surgical gauze secured in place with hypo-allergenic tape. The entire test area was further covered with polyethylene film (occlusive) also secured to the trunk of the animal with tape.

Test article was allowed to remain in contact with the skin for 24-hr, after which the occlusive dressing was removed. Although the endpoint is lethality, animals were observed for other signs of toxicity (e.g. tremors/convulsions, diarrhea, salivation, somatomotor activity) as well as for skin irritation (Refer to Table 5 for characteristic acute toxic effects associated with agent, oxidant, or solvent). Cage-side observations were continuous for 8 hours, and the periodicity of subsequent observations during the initial 24-hour post-exposure period was based on the condition of the animals. The animals were observed for an additional 13 days to permit a full evaluation of the reversibility or irreversibility of effects. Animals were euthanized and disposed of on completion of the 14-day observation period.

2.4.2.2 LD50 Test.

DOT regulations (1990, 1993) require the assessment of a test substance to "packing groups" based on the LD_{50} test[8]. A "statistically valid" number of rabbits (albino, unspecified age and sex) are dosed for 24-hr and survivors observed for 14-days. Animal preparations would be the same as that described for the limit test.

Department of Transportation
Packing Designation[1]

Packing Group	Dermal Toxicity LD50 (mg/kg)
I	<40
II	>40 <200
III	>200 <1000

1 Federal Regulation (49 CFR Part 107, 21 Dec 1990)

[8] A definitive LD_{50} not required, most regulatory agencies are satisfied with an estimated LD_{50} value or an approximate lethal dose. Newer regulatory guidelines allow or even promote study designs where only an estimate of a lethal dose is obtained.

LD_{50} tests on wastestreams were deemed unnecessary taking into account collective toxicity data comprised of (1) results of the limit tests on wastestreams, (2) existent LD_{50} data on oxidizers and solvents (refer to Table 6), and (3) the residual levels (ppm) of each agent detected in the wastestreams (refer to Table 8). Vesicant agents at ppm levels are generally considered not to elicit overt toxic signs (refer to reviews on systemic action of blistering agents: HD (Smith, 1943b; Smith et al., 1944; Anslow and Houck, 1946); HN (Cope et al., 1946; Anslow and Houck, 1946); and (Gates et al., 1946). Furthermore, the decision not to proceed with LD_{50} testing is consistent with the EPA tiered approach to acute toxicity testing.

Table 5. Acute Toxic Effects Associated with CAIS Agents and RRS Neutralization Chemicals and Solvents

Compound	Toxicologic Endpoints Indicative of Acute Systemic Intoxication[a]	Dermal LD50
Agents		
Sulfur Mustard (HD)	anorexia, diarrhea, vomiting, salivation, cachexia, hyperpnea, tremors/convulsions, prostration, death	≈ 40 mg/kg (highly toxic)[b,c]
Nitrogen Mustard (HN) (HN-1, HN-3)	anorexia, diarrhea, vomiting, salivation, cachexia, hyperpnea, tremors/convulsions, prostration, death	≈ 15 mg/kg[d] (highly toxic)
Lewisite (L)	anorexia, nausea, vomiting, pulmonary edema, hemodynamic shock, prostration, death	≈ 5 mg/kg[e] (highly toxic)
Oxidants		
m-chloroperoxybenzoic acid (m-cpba)	localized irritant effects (respiratory tract, skin) systemic effects probably minimal	> 20 g/kg[f] (practically non-toxic)[g]
1,3-dibromo-5,5 dimethyl-hydantoin (Brom-55p)	localized irritant effects (respiratory tract, skin) dyspnea (may be systemic) systemic effects probably minimal	> 20/g/kg (practically non-toxic)
1,3-dichloro-5,5 dimethyl-handantoin (DCDMH)	localized irritant effects (respiratory tract, skin), dyspnea (may be systemic) somnolence and tremors indicative of systemic poisoning	> 20 g/kg (practically non-toxic)

Table 5. (Continued)

Compound	Toxic Effects Indicative of Acute Systemic Intoxication[a]	Dermal LD50
Solvents		
Chloroform ($CHCl_3$)	narcosis, CNS depression, pulmonary edema, arrhythmia, kidney/liver necrosis, prostration, death	> 20 g/kg (practically non-toxic)
t-butyl alcohol (t-BuOH)	narcosis, ataxia, CNS depression, death	(-)[h]
Sulfolane	CNS depression, tremors/ convulsion, respiratory depression, death	3.2 g/kg[i] (slightly toxic)

[a] In general, manifestation of systemic toxicity depends on the severity as well as the route of exposure.

[b] HD elicits a local vesicant action on skin, damage to eyes and mucosal lining of respiratory tract with appreciable systemic toxicity [dermal LD50 ≈40 mg/kg (Anslow and Houck, 1946)]. At LD50 doses or above, characteristic biologic actions are manifest on the nervous system - readily elicited on i.v. dosing less readily on administration by other routes.

[c] HD is considered highly toxic] per toxicity classification outlined by Hodge and Sterner (1943).

[d] Physiological actions are similar to HD. In addition to local vesicant and strong irritant action, HN possesses appreciable independent systemic toxicity [dermal LD50 ≈ 15 mg/kg (Smith, 1943a) Anslow and Houck (1946) categorized the total systemic injury as moderate following dermal application of nitrogen mustard (LD50 dose) and as mild after dermal dosing with sulfur mustard (LD50 dose).

[e] L is a highly toxic systemic poison [dermal LD50 ≈ 5 mg/kg (Cameron et al, 1946)in addition to its vesicant/irritant properties.

[f] The dermal LD50 estimate is based on mouse data. Although rabbit data is not available, one may postulate that the rabbit dermal LD50 would be lower since the rabbit skin is more sensitive to the injurant action of toxicants than mouse/rat.

[g] The toxicity classification (practically non-toxic) according to Hodge and Sterner (1949) would still be relevant.

[h] Dermal data not available - the rabbit oral LDLo is 4.5 g/kg. The material according to the toxicity classification of Hodge and Sterner (1943) would probably be considered as slightly toxic by either oral or dermal route of exposure.

[i] Sulfolane, per the toxicity classification scheme of Hodge and Sterner (1943), may be classified as practically non-toxic. Considering that the LD50 (3.2 g/kg) is at the low end of the range of values for practically non-toxic materials, sulfolane could conservatively be considered as slightly toxic by the dermal route.

2.4.2.3 Rabbit Dermal Irritation Studies.

The primary dermal irritation (PDI) potential of process chemistry wastestreams, of oxidizer/solvent systems, and of solvents were conducted in accordance with DOT guidelines[9] (CFR, 1993). Adult rabbits (sufficient numbers [≥6] to allow for adequate assessment of dermal toxicity potential) were treated with "test article" as indicated in Table 4. It should be emphasised that the investigators,

[9]DOT guidelines similar/identical to EPA and OECD guidelines (Meyers and DePass, 1993).

consistent with the national trend, adhered to the principle of animal use minimization expoused initially by Russell and Burch (1959). Reduction in animal usage was most apparent in the skin irritation testing of modified "Red" and "Charcoal" process chemistry components where the solvent and oxidant systems were common to both process chemistries. Test material (0.5 ml) was applied to the test site (clipped dorsal lumbar area) and the dose site occluded[10] - each animal served as its own control. The occlusion-exposure period was for four hours.
With the exception of the Brom-55p wastestream,[11] the treated areas (neutralized CAIS or agent controls) were not deconned. The degree of irritation was read and scored according to the grades in Table 7. Rabbits were examined for indications of erythema and edema at 30-60 minutes, at 24, 48, and 72 hours after patch removal. Also, any serious lesions and/or other indications of erythema and edema at 30-60 minutes, 24, 48, and 72 hour toxic effects were noted. Observations were extended to 14 days (7 and 14-day evaluations) to evaluate the reversibility or irreversibility of the effects observed.

2.5 Data Analysis.

Statistical evaluation of the scored data was based on the following statistical measures: (1) Kruskal-Wallis test (non-parametric analog of the one-way ANOVA); (2) Mann-Whitney test (non-parametric rank/sum test); and (3) Student-Newman-Keuls test (pairwise multiple comparison test). The Mann-Whitney (Daniel, 1983) and Kruskal-Wallis (Hintze, 1989) tests show that the non-parametric Draize erythema and edema scores differ significantly ($p<0.05$) from each other. The Newman-Keuls test, as a multiple comparison procedure, identifies treatment group(s) that differ significantly ($p<0.05$) from each other. The above statistical measures were available through the statistical program Sigma Stat® by Jandel Scientific Software, Version 1.03.

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[10] **The use of occlusive dressing is a severe test.**

[11] **Because the HD level of the Brom-55p wastestream was above 100ppm,treated sites were deconned with 5 % sodium hypochlorite solution. The treatment with decon, applied for 30 seconds and immediately rinsed with water, resulted in no skin irritation.**

Table 6. Synopsis of Dermal Toxicity Data for CAIS Agents, Agent Degradation Products[a], RRS Oxidants and Solvents

Compound	Dermal Toxicity[b] (LD_{50}/LDLo/TDLo)	References	Skin Effects (Irritation, Vesication)[b]	References
AGENTS				
HD [bis(2-chloroethyl)sulfide]	LD_{50} (40-100 mg/kg)	Anslow & Houck (1946)	Severe irritant/escharotic, severe vesicant	Marshall & Williams (1921); Gates & Moore (1946); Renshaw (1946)
L [dichloro(2-chlorovinyl)arsine]	LD_{50} (5-6 mg/kg)	Cameron et al. (1946); Gates et al. (1946)	Severe irritant/escharotic, severe vesicant	Gates et al. (1946)
HN-1 [bis(2-chloroethyl)ethylamine]	LD_{50} (15-20 mg/kg)	Smith (1943a); Anslow & Houck (1946)	Severe irritant/escharotic, severe vesicant	Cope et al. (1946); Renshaw (1946)
HN-3 [tris(2-chloroethyl)amine]	LD_{50} (5-20 mg/kg)	Smith (1943d); Anslow & Houck (1946)	Severe irritant/escharotic, severe vesicant	Cope et al. (1946); Renshaw (1946);
OXIDIZED DERIVATIVES				
HD sulfoxide	(-)[c]	(-)[c]	Irritant, non-vesicant	Marshall & Williams (1921); Lawson & Dawson (1927); Young et al. (1944)
Sulfoxide, 2-chloroethyl vinyl	(-)[d]	(-)[d]	Irritant, non-vesicant	Thomson et al. (1945)
Divinyl sulfoxide	(-)[e]	(-)[e]	Irritant, non-vesicant	Fuson et al. (1943); Young et al. (1944) Thomson et al. (1945)
HD sulfone	(-)[f]	(-)[f]	Irritant/escharotic, vesicant	Marshall & Williams 1921); Young et al. (1944)

Table 6. (Continued)

Compound	Dermal Toxicity[a] (LD_{50}/LDLo/TDLo)	References	Skin Effects (Irritation, Vesication)[b]	References
OXIDIZED DERIVATIVES (Cont.)				
Sulfone, 2-chloroethyl vinyl	(-)[g]	(-)[g]	Irritant/escharotic, vesicant	Young et al. (1944) Thomson et al. (1945)
Divinyl sulfone	LD_{50} (≈ 20 mg/kg)	Smyth et al. (1962)	Irritant/escharotic, vesicant	Young et al. (1944); Thomson et al. (1945)
HN-1 oxide	(-)[h]	(-)[h]	(-)[p]	(-)[p]
HN-3 oxide	(-)[i]	(-)[i]	(-)[p]	(-)[p]
Lewisite oxide	(-)[j]	(-)[j]	Irritant/escharotic, vesicant	Young et al. (1944); Thomson et al. (1945)
2-chlorovinylarsonic acid	(-)[k]	(-)[k]	Irritant, non-vesicant	Young et al. (1944); Thomson et al (1945)
2-chlorovinylarsonous acid	(-)[l]	(-)[l]	Irritant, non-vesicant	Cameron et al. (1946)
OXIDIZERS				
DCDMH	LD_{50} (>20 g/kg)	EPA 8EHQ0281-0382; EPA 88-8100-228 (cited in RTECS)	Severe irritant	EPA 8EHQ0281-0382; EPA #88-8100-173 (cited in RTECS)
Brom-55p	LD_{50} (>20 g/kg) LDLo (20 g/kg)	EPA 8EHQ0581-0382; EPA #88-8100-173 and 88-8100-228 (cited in RTECS)	Severe irritant	EPA #88-8100-173; and 88-8100-228 (cited in RTECS)

Table 6. (Continued)

Compound	Dermal Toxicity[a] (LD50/LDLo/TDLo)	References	Skin Effects (Irritation, Vesication)[b]	References
OXIDIZERS (Cont.)				
m-cpba	TDLo (21 g/kg)[m]	Sax(1984)	Severe irritant	J.T. Baker, Inc., MSDS (1994)
SOLVENTS				
Chloroform	LD_{50}(>20 g/kg)	NTIS AD-A062-138 (cited in RTECS)	Mild irritant	Guido and Martins (1988)
t-butyl alcohol	(-)[n]	(-)[n]	Mild irritant	Oettel (1936)
Sulfolane	LD_{50} (≈ 3 g/kg)	Smyth et al. (1969)	Irritant[o]	J.T. Baker, Inc., MSDS (1994)

[a] Toxicity profiles of agent degradation products are given in Appendix B of this report.
[b] Rabbit as animal model unless otherwise indicated. Tests for irritancy based on animal and/or human studies. Test for vesicant action of agents conducted on human subjects.
[c] Mouse s.c. LD_{50} (>25 mg/kg) [Anslow and Houck (1946)].
[d] Rat oral (100 mg/kg, mortality 1/1) [Young et al., 1944]
[e] Mouse s.c. LD_{50} (>25 mg/kg) [Anslow ad Houck (1946)].
[f] Mouse s.c. LD_{50} (>25 mg/kg) [Anslow and Houck (1946)].
[g] Acute toxicity undetermined.
[h] Mouse i.p. LD_{50} (50-100 mg/kg) [Bergmann and Fruton (1943); Stahmann and Bergmann (1946a)].
[i] Mouse i.p. LD_{50} (2-5 mg/kg) [Bergmann and Fruton (1943); Stahmann and Bergmann (1946a)].
[j] Mouse s.c. (mortalities: 2 mg/kg (0/5); 5 mg/kg (5/5); 10 mg/kg (5/5) [Young et al. (1944)].
[k] Mouse i.p. [mortalities: (1000 mg/kg 10/10; 500 mg/kg 0/10] (Young et al., 1944)
[l] Reported as hightly toxic, details not given (Cameron et al., 1946)
[m] Mouse skin TDLo (21 g/kg).
[n] Rabbit oral LDLo (4.5 g/kg) [RTECS].
[o] Brown et al. (1966) reported sulfolane as free of skin irritant effects.

Table 7. Grading Values for Skin Reaction (Draize Scoring System for Skin Irritation)[a]

Reaction	Score
Erythema and Eschar Formation	
No erythema	0
Very slight erythema (barely perceptible)	1
Well-defined erythema	2
Moderate to severe erythema	3
Severe erythema to slight eschar formation	4
Edema Formation	
No edema	0
Very slight edema (barely perceptible)	1
Slight edema (definite raising)	2
Moderate edema	3
Severe edema (extending beyond area of exposure)	4

[a] **Method currently used still basically that proposed by Draize et al. (1944) and Draize (1965) as modified (16 CFR, 1987). Currently, it exists legislatively under provisions of the Federal Hazardous Substance Act.**

3. RESULTS

3.1 Chemistry.

3.1.1 Process Chemistries.

The chemical agents (HD, HN, L) are components of CAIS that are chemically neutralized ("detoxified") on reaction with treatment reagent. The selection of a particular process chemistry (designated as "Blue", "Red", or "Charcoal" process) is dependent on whether the agent is neat material (as in the case of sulfur mustard), in solution ($CHCl_3$ as solvent), or absorbed on charcoal. As stated previously, chemistry methodologies were driven not only by the need for the rapid and effective chemical degradation of agent but also by development of a neutralization process that minimizes health risks and maximizes safety.

3.1.1.1 Blue Process Chemistry.

The initial process chemistry for the neutralization of

CAIS containing neat HD utilized 1,3-dibromo-5,5-dimethyl hydantoin (Brom-55p) as oxidizer in sulfolane/3% water, and the modified process chemistry utilized DCDMH as oxidizer in chloroform/t-butyl alcohol/3% H_2O. Chemical neutralization of sulfur mustard via Brom-55p resulted in a mixture of about a dozen components. Products formed in the Brom-55p-mediated reaction included sulfides[12] (area %: 10.7%) and sulfoxides (area %: 82.7%) with agent (HD) residue concentration of ≈100 ppm. Unknown content of the Brom-55p wastestream was nil.

The DCDMH reaction resulted in comparable sulfides content (area %: 16.8%) and somewhat diminished sulfoxides content (area %: 71.2.%) compared to the Brom-55p mediated neutralization. Sulfur mustard concentration was below 50 ppm in the DCDMH wastestream. Mustard sulfone content for both Brom-55p and DCDMH wastestreams was below 2% [area % of the total ion chromatogram (TIC)]. The 1,3-dichloro-5,5-dimethyl hydantoin (DCDMH)-mediated reaction resulted in a much more complex mixture having a far greater proportion of vinyl **(C=C)** containing degradation products (area %: 77.6%). Vinyl containing compounds are generally associated with greater toxicity than their corresponding saturated analogs. Unknowns in the DCDMH-mediated reaction accounted for 7.7%, as determined from the TIC, which is in marked contrast to the Brom-55p mediated neutralization where all wastestream components were verifiable via GC/MS analyses. Information on agent residue level and product characterization of "Blue Process" wastestreams are highlighted in Table 8 and results provided in greater detail in Appendix G, Tables G-1 and G-2.

3.1.1.2 <u>Red Process Chemistry</u>.

The initial process chemistry utilized m-chloroperoxy-benzoic acid (m-cpba) as oxidant, whereas the modified process chemistry used DCDMH as oxidizer. The neutralization reactions (initial and modified "Red Process" chemistries) resulted in fairly complex product solutions (mixtures) containing various products, by-products and residual amounts of unreacted agent (HD, HN-1, and L). Sulfur mustard and lewisite levels were below detection limits. Also, pertaining to agent residue levels, it is noteworthy that the DCDMH-mediated reaction was more effective in neutralizing nitrogen mustard, than m-cpba [conc: $\leq$ 50 ppm versus 157 ppm, DCDMH and m-cpba mediated reactions respectively]. Both treatment reagents resulted in the oxidation of sulfur mustard to sulfoxide and sulfone analogs. Striking results were obtained in the DCDMH-mediated reaction with respect to HD sulfone content. There was marked reduction in HD sulfone content in the DCDMH-mediated reaction (area %: 0.5%) compared to the high HD sulfone levels (area %: 20.9%) generated in the m-cpba wastestream. Substitution and elimination reactions also occurred which produced both chlorinated and vinyl sulfoxides.

[12] **Objective was to minimize sulfides, generally regarded as highly toxic in comparison to sulfoxides. Preliminary analysis on the first set of "Blue Process" wastestreams indicated that the total sulfides content in DCDMH-mediated degradation of HD was markedly reduced compared to sulfides content of Brom 55-p mediated degradation of agent. These early findings, however, were not corroborated in subsequently generated and analyzed wastestreams.**

Nitrogen mustard[13] [**HN-1 was used in the m-cpba reaction (initial "Red Process" chemistry); HN-3 was used in the DCDMH reaction (modified "Red Process" chemistry)**] was converted to the amine oxide, which can undergo subsequent conversion to cyclic intermediates. Lewisite was oxidized to chlorovinylarsonic acid (major product) and other products. Also, there was a preliminary indication that the alkoxyamine levels were also reduced in the DCDMH-mediated reaction. The level of unknowns did not exceed 6.5% (area % of the TIC) for both m-cpba and DCDMH-mediated reactions. The reader is referred to Table 8 which highlights agent residue levels and product characterization of "Red Process" wastestreams - detailed characterization data are provided in Appendix G, Tables G-3 and G-4.

3.1.1.3 Charcoal Process Chemistry.

The process chemistries for the neutralization of CAIS containing agent on charcoal resulted in the formation of complex product solutions - highly complex in the modified "Charcoal Process" chemistry - containing oxidized agent products and chlorinated analogs. GC-MS analysis of the wastestream from the initial "Charcoal Process" chemistry (DCDMH/$CHCl_3$) revealed agent residue levels as follows: HD, HN-1 ($\leq$ 50 ppm), L (>>200 ppm). The addition of t-butyl alcohol, as a co-solvent, to the reaction with L (designated as the modified "Charcoal Process" chemistry) resulted in appreciable reduction in L residue levels (<< 200 ppm). Despite the benefits of a much lowered residual L content ($\leq$ 50 ppm), the modified "Charcoal Process" chemistry produced a highly complex multi-component mixture which did not lend itself to good analytical characterization. A summary of agent residue levels and product characterization of "Charcoal Process" wastestreams is highlighted in Table 8 and in greater detail as provided in Appendix G, Table G-5.

3.1.2 Analytical Results.

From the onset, it must be stated that the wastestreams were complex, reactive mixtures (particularly exemplified by the exceeding complexity of the "Charcoal Process" wastestream), which pushed the existent analytical methodologies (GC/MS) to the limits of sensitivity, mixture analysis capability, and structural elucidation. Wastestreams (pooled in the case of "Red" and "Charcoal" process chemistries) from the neutralization reactions were analyzed by GC-MS spectroscopy. Instrumentation was operated in the electron ionization (EI) mode for residual agent analyses and in the CI/EI modes for wastestream component analyses. With the GC/MS/EI system, the Method Quantitation Limit (MQL), the concentration level that can be quantitatively reproduced for agent (HD, HN, or L), was 50 μg/ml (50 ppm).

[13] Ideally, the intent was to use HN-1 for all "Red Process" chemistry studies; however, scarcity of HN-1 required the use of HN-3 in the modified red process chemistry. Both forms of nitrogen mustard (HN-1 or HN-3) are thought to react similarly with oxidizing material although Franke (1967) makes the point that HN-3 is extremely stable to oxidizing agents. HN-1 and HN-3 have essentially similar biological activity (Daily et al. (1944); Gates and Moore (1946); Cope et al. (1946)).

Table 8. Comparison of Agent Residue Levels, Major Products/ By-products and Unknowns in Wastestreams

Process Chemistry

(Blue Process)

Component	Initial area %/ppm	Modified area %/ppm[a]
HD	(109 ppm)	(< 50 ppm)
HD sulfoxide	4.8%	0%
HD sulfone	1.5%	1.2%
Divinyl sulfone	(-)	0.3%
Sulfides[c]	10.7%	16.9%
Sulfoxides[c]	82.7%	71.2%
Unknowns	0%	7.7%
Other	(-)	2.7%

(Red Process)

	Initial area %/ppm	Modified area %/ppm
HD(< 50 ppm)	(< 50 ppm)	(< 50 ppm)
HN1	(157 ppm)	(< 50 ppm)[b]
L	(<200 ppm)	(102 ppm)
HD sulfone	20.9%	0.5%
Sulfides[c]	(-)	(-)
Sulfoxides[c]	16.7%	59.5%
Unknowns	6.4%	3.2%
Other	46.9%	30.6%

(Charcoal Process)

	Initial area %/ppm	Modified area %/ppm
HD	(≥ 50 ppm)	(< 50 ppm)
HN1	(≥ 50 ppm)	(< 50 ppm)
L	(200 ppm)	(<< 200 ppm)
HD sulfone	(-)	(-)
Sulfides[c]	(-)	(-)
Sulfoxides[c]	(-)	(-)
Unknowns	(-)	50.3%

[a] **Agent levels (ppm); products/by-products and unknowns (area%).**
[b] **HN3 in place of HN1**
[c] **(Saturated and Unsaturated).**
(-) **Not Detected**

In instances were GC/MS/CI-based analyses were used, the MQL for agent was greater than 50 μg/ml [i.e. 100 μg/ml (100 ppm) for sulfur mustard]. Only numerical values for agent levels are used in this report if agent levels are at or above the MQL, otherwise agent residue levels are reported as ≤ 50 ppm. The analytical data was based on the best GC/MS methodologies available at the time for the analysis of components in complex matrices, and every reasonable attempt was made to overcome technical difficulties arising from such circumstances as detection interferences; for example, presence of chlorovinyl-arsonic acid (CVAOA) which hampered GC/MS identification of lewisite degradation products. Most of the reaction products of HN could not be detected per the current GC-MS methodology. Results of the GC/MS/CI analyses of the wastestreams for identification of reaction products are presented as relative peak areas [area % from the Total Ion Chromatogram (TIC)]. Agent residue levels, major

product/by-product content, and unknowns in the wastestreams is summarized in Table 8. Detailed product profiles for the various wastestreams appear in Appendix G.

3.2 Toxicology:

3.2.1 Skin Irritation Testing (4-Hr Occluded):

3.2.1.1 Brom-55p Reaction (Initial Blue Process Chemistry):

The wastestream from the Brom-55p reaction with sulfur mustard as well as the solvent and oxidizer/solvent system were evaluated for skin irritation potential following 4-hr occluded exposure to test article. The irritation response data for each individual animal for each observation period after treatment with the particular test article is given in Appendix H. The incidence of skin injury (erythema/edema) on exposure to each "test article" is provided in Table 9. Sulfolane, the solvent for the Brom-55p reaction, was tested for skin effects (see Appendix H, Table H-1). The first indication of skin response (erythema, grade 1) was noted in 1/4 treated rabbits at 72 hrs after removal of the occlusive dressing (patch). No edema was seen at the 72-hr observation nor at any other observation period following exposure to test article. At 7 days after patch removal, severe erythema (grade 4) was noted in 1/4 rabbits exposed to sulfolane - the degree of injury may have been due to a self-inflicted injury rather than chemical-induced lesions since sulfolane is reported to have minimal skin irritating properties. Some evidence of recovery (decreased eschar) to "sulfolane- induced" injury was confirmed at the 14-day post-exposure period. Animals treated with the oxidizer/solvent system(Brom-55p/sulfolane), (refer to Appendix H, Table H-2) exhibited skin-irritation (slight erythema) at 30-60 mins following removal of the occlusive dressing. Increased severity of the skin response to test article was noted at the 24-hr observation period when animals exhibited well-defined to moderate erythema with slight to moderate edema. Severe erythema and edema were manifest in 2/4 treated animals at 48 hours. At 72 hours after treatment with oxidizer/solvent, indications of recovery were evident (1/4 animals no erythema; 2/4 animals no edema). At 7 days post-exposure, there were indications of continued recovery to the skin-damaging effects of test material. At 14 days post-exposure, evidence of continued recovery as the two rabbits, exhibiting the most severe skin reaction to Brom-55p/sulfolane, were much improved.

The wastestream from the Brom-55p reaction with sulfur mustard was assessed for cutaneous injury (non-vesicant) following a 4-hour occlusive exposure (see Appendix H, Table H-3). The wastestream contained ppm levels of HD. Prior to describing the results from the wastestream and sulfur mustard experiments, it should be stated that in regard to vesicants, a vesicant may produce skin injury varying in severity from transient erythema to coagulation and necrosis of the epidermis/ dermis depending on the severity of exposure and tissue susceptibility. Description of the

Table 9. Incidence of Skin Injury on Exposure (4-hr) to Brom-55p Neutralized HD, or Oxidizer/Solvent System[a]

Treatment Group	Incidence		
	Erythema[b]	Edema[c]	Time
Sulfolane	0/4	0/4	30-60 mins[d]
	0/4	0/4	24-hr
	0/4	0/4	48-hr
	1/4	0/4	72-hr
	1/4	0/4	7-day
	0/4	0/4	14-day
Oxidizer/Solvent[e] (Brom-55p/Sulfolane)	4/4	0/4	30-60 mins
	4/4	3/4	24-hr
	4/4	4/4	48-hr
	3/4	2/4	72-hr
	3/3	2/4	7-day
	1/4	3/4	14-day
Wastestream[f]	4/4	0/4	30-60 mins
	4/4	4/4	24-hr
	4/4	4/4	48-hr
	4/4	4/4	72-hr
	4/4	3/4	7-day
	1/4	3/4	14-day
HD[g]	4/4	0/4	30-60 mins
	4/4	4/4	24-hr
	4/4	2/4	48-hr
	4/4	2/4	72-hr
	2/4	1/4	7-day
	0/4	1/4	14-day

[a] Cutaneous injury was assessed following a 4-hr occluded exposure to test article (0.5 ml). Visual assessment of the degree of erythema/edema was made per the scoring system of Draize et al. (1944).
[b] Redness of the skin (severity scale of 0-4).
[c] Localized swelling (severity scale of 0-4).
[d] Initial observations were made 30-60 minutes after removal of the occlusive dressing (patch).
[e] Decon mixture consisted of 0.55M Brom-55p in sulfolane with 3% water.
[f] Wastestream contained oxidizer/solvent system, products and by-products of HD degradation, and residual levels (ppm) of HD.
[g] Amount of HD topically applied (0.5 ml of an isopropanol solution containing 109 ppm HD)

sequence of pathologic changes following application of blistering agent has been reported for humans (Renshaw, 1946), rabbit (McMaster and Hogeboom, 1945), and rat (Sullivan, 1942). At the initial observation (30-60 mins after patch removal), 3/4 rabbits manifested skin irritation (well-defined erythema); however, edema was not noted. At 24 hours, both erythema and edema were observed - the edema was moderate in degree (3/4 rabbits). The skin response increased in severity evidenced by a grade 3 erythema (moderate-to-severe) in 2/4 treated rabbits observed at 48 hrs post-exposure, and an erythema response (severe) noted in 3/4 rabbits examined at 72 hours post-exposure. All animals evaluated at 7 days after dosing still exhibited severe erythema whereas the edema associated with the chemical treatment had subsided (2/4). At 14 days, healing of damaged skin was progressing.

The cutaneous injury (non-vesicant) following a 4-hour exposure to sulfur mustard, as a positive control, was also evaluated (refer to Appendix M, Table M-1). At 30-60 minutes after patch removal, a non-edematous erythema (grade 2, well-defined) was observed in 4/4 HD-treated rabbits. At 24 hours post-exposure, a grade 2 erythema was noted concomitant with a grade 1 edema in all treated animals. The 48 hour observations, coupled with the 72 hour observations, provided indication that the animals were recovering from the skin- damaging effects of HD (at 48 hr: 2/4 animals with grade 1 erythema; 2/4 animals with no edema; at 72 hr: 3/4 animals with grade 1 erythema; 2/4 animals with no edema). At 7 days post-exposure, 2/4 animals exhibited erythema and 1/4 animals manifested some edema. At 14 days, recovery was essentially complete. Comparison to skin reactions with other CAIS wastestreams and oxidant/solvent systems is highlighted in Tables 14 and 15.

3.2.1.2 DCDMH Reaction (Modified Blue Process Chemistry):

The wastestream resultant from the chemical neutralization of HD via DCDMH in $CHCl_3$/t-BuOH was tested for skin injurant potential.The incidence of skin injury is presented in Table 10, and detailed results given in Appendix I.. Exposure to the above wastestream resulted in moderate to severe erythema (grades 3-4) with slight edematous reaction (grade 2). The erythema component peaked at 72-hr post-exposure which did not subside until 14-day post-exposure. The edema had subsided (Grade 0) by the 7th day post-exposure.

Table 10. Incidence of Skin Injury on Exposure (4-hr) to DCDMH-Neutralized Sulfur Mustard (HD)[a]

	Incidence		
Treatment Group[b]	**Erythema[c]**	**Edema[d]**	**Time[e]**
Wastestream	6/6	6/6	30-60 mins
	6/6	6/6	24-hr
	6/6	6/6	48-hr
	6/6	6/6	72-hr
	6/6	0/6	7-day
	6/6	0/6	14-day

[a] Skin injury was evaluated after a 4-hr occluded exposure to "test article" (0.5 ml). Visual assessment of the degree of erythema and edema was made per the scoring system of Draize et al. (1944).
[b] Oxidizer/solvent system (DCDMH/$CHCl_3$/t-BuOH) same as for "Modified Red" process - refer to Table 12 for results.
[c] Redness of the skin (scale of severity 0-4).
[d] Localized swelling (scale of severity 0-4).
[e] Initial observations were made 30-60 minutes after removal of the occlusive patch.

3.2.1.3 m-cpba Reaction (Initial Red Process Chemistry):

The wastestream generated by the reaction of m-cpba and CAIS containing HD, HN or L in $CHCl_3$, as well as the oxidant/ solvent system, were tested for skin irritation (results are tabulated in Appendix J). The incidence of skin injury is highlighted in Table 11. Chloroform was tested for irritant response in the assessment of

the DCDMH-mediated wastestream/component studies (refer to Appendix K, Table K-1 and Table 12). Exposure to the oxidant/solvent system (m-cpba/$CHCl_3$) resulted in substantial cutaneous injury (see Appendix J, Table J-1.)' Animals observed 30-60 mins after patch removal exhibited a grade 2 erythema in 6/6 animals, and the degree of edema varied from grade 2 to grade 4. Varied degrees of erythema were noted at 24 hours; however, by 48 hours post-exposure severe erythema (grade 4) was evident in 6/6 rabbits - the visible lesion having plateaued. At 7 days post-exposure, there was some indication that the erythema response had subsided although 3/6 rabbits still exhibited a severe degree of erythema and edema was still evident. By the 14th day post-exposure, the degree of erythema had subsided such that only 1/6 rabbits exhibited severe erythema. Edema was still noticeable at 14 days post-exposure.

The m-cpba generated wastestream from the neutralization of HD, HN, and L produced skin injury that was characterized by necrotic encrusted lesions. At 24-hrs post-exposure, moderate-to-severe and severe grades of erythema were observed with severe edema which evolved to severe edematous erythema in 10/10 rabbits at 48 hours. The 72 hr observations confirmed that severe epidermal damage had occurred. At 7 days post-exposure, despite indications that the edema had subsided, deep eschar was noted with necrotic encrusted lesions - there was no indication of healing. At the 14 day post-exposure observation, some improvement was noted in the condition of the rabbits; however, in general, recovery to the m-cpba wastestream induced skin damage was incomplete. Refer also to Tables 14 and 15 for comparison of skin reaction to other wastestreams and oxidant/solvent systems.

Table 11. Incidence of Skin Injury on Exposure to m-cpba Neutralized Agent (HD, HN, or L) or Oxidizer/ Solvent System[a]

Treatment Group	Incidence		
	Erythema[b]	Edema[c]	Time
Oxidant/Solvent[d]	6/6	6/6	30-60 mins[e]
	6/6	6/6	24-hr
	6/6	6/6	48-hr
	6/6	6/6	72-hr
	6/6	6/6	7-day
	6/6	5/6	14-day
Wastestream[e]	(-)[f]	(-)[f]	30-60 mins
	10/10	10/10	24-hr
	10/10	10/10	48-hr
	10/10	10/10	72-hr
	10/10	10/10	7-day
	10/10	10/10	14-day

[a] Cutaneous injury was assessed following occluded exposure to "test article". Visual assessment of the degree of erythema/edema was made per scoring system of Draize et al. (1944).
[b] Redness of the skin (severity: scale of 0-4).
[c] Localized swelling (severity: scale of 0-4).
[d] Decon mixture consisted of m-cpba in $CHCl_3$/t-BuOH. Exposure was 4-hrs to 0.5 ml test material per skin irritation test paradigm.
[e] Exposure duration to wastestream was for 24-hr. These observations were part of the 24-hr lethality screen conducted on animals dosed with 0.5 ml/kg of test material. Conditions of test much more severe than the conventional irritation test.
[f] The initial observations were 24-hr post-exposure as per the lethality screen/limit test paradigm.

3.2.1.4 DCDMH Reaction (Modified Red Process Chemistry):

The wastestream from the DCDMH-mediated neutralization of CAIS, containing HD, HN, or L in chloroform, as well as the oxidant/solvent system and solvents, were tested for skin irritation. The irritation response data is presented in detail in Appendices K. The incidence of skin injury following exposure to each "test article" is given in Table 12. The solvents (chloroform, t-butyl alcohol) were tested individually and as a mixture for skin irritation potential. A 4-hr occlusion exposure to t-butyl alcohol produced no skin irritation in rabbits. Exposure to chloroform resulted in cutaneous injury(refer to Appendix K, Table K-1), noted initially at 30-60 mins post-exposure as very slight erythema (grade 1) and as slight edema (grade 2), characterized at its peak (48-hr observation), by well-defined erythema (grade 2) and slight to moderate edema (grade 2 (3/6); grade 3 (3/6)). At 72 hours post-exposure, the erythema and edema had subsided [(grade 1) erythema (3/6); no edema (6/6)]. The absence of erythema and edema at the 7 and 14-day observations indicated complete reversal of skin damage. Animals exposed to the co-solvent ($CHCl_3$/t-BuOH) manifested skin irritancy (refer to Appendix K, Table K-3 and Table 12). The initial signs of skin irritation consisted of mild erythema (grade 1) in 5/6 animals and as very slight edema (grade 1) in 4/6 treated animals 30-60 minutes after patch removal. The most severe skin reaction to treatment was noted at the 48-hr post-exposure observation when 5/6 treated rabbits manifested well-defined erythema (grade 2)and edema [slight (3/6); moderate (3/6)]. Indication of reversibility to co-solvent induced skin injury was noted at 72 hours. No skin pathology was noted at 7 days postexposure - indicative of complete recovery. The 14-day observation corroborated the findings previously noted at 7 days post-exposure.

Exposure to the oxidant/solvent system resulted in considerable skin injury which had not resolved even at 14 days post-exposure (refer to Appendix K, Table K-4 and Table 12). Severe (grade 4) erythema and severe (grade 4) edema were noted 24 hours after patch removal. The condition of the treated sites had not improved at 72 hours and in fact had deteriorated, evidenced by the presence of deep well-defined eschar. At 7 and 14 day post-exposure, animals exhibited epidermal necrosis and erosion with severe edema. Re-epithelialization (healing) was not evident at 7 or at 14 days.

Occluded exposure (4-hr) to wastestream generated from the reaction of DCDMH and CAIS, containing HD, HN or L in chloroform, resulted in cutaneous injury (see Appendix K, Table K-5). Erythema (very slight to well-defined) with intense edema were characteristic features of the 30-60 min observation after patch removal. At 24-hr post-exposure well-defined erythema (5/6 rabbits) with slight/moderate edematous reaction were noted.The erythema had not plateaued over the course of the next two days; however, the edematous component of the skin reaction to test material had subsided to very slight/slight degrees of severity. The late lesion (7 days) presented a severe (grade 4) erythema with no indication of edema. At 14 days post-exposure, severe erythema presented with no manifestation of edema. For comparative purposes, skin reactions following treatment with the various wastestreams and oxidant/solvent systems are presented in Tables 14 and 15.

Table 12. Incidence of Skin Injury on Exposure (4-hr) to DCDMH-Neutralized Agent (HD, HN, L) or Oxidizer/Solvent System[a]

Treatment Group	Incidence		
	Erythema[b]	Edema[c]	Time
Chloroform	6/6	6/6	30-60 minS[d]
	6/6	6/6	24-hr
	6/6	6/6	48-hr
	6/6	0/6	72-hr
	0/6	0/6	7-day
	0/6	0/6	14-day
t-butyl alcohol	0/6	0/6	30-60 mins
	0/6	0/6	24-hr
	0/6	0/6	48-hr
	0/6	0/6	72-hr
	0/6	0/6	7-day
	0/6	0/6	14-day
Chloroform/t-butyl alcohol	6/6	5/6	30-60 mins
	6/6	6/6	24-hr
	6/6	6/6	48-hr
	6/6	0/6	72-hr
	0/6	0/6	7-day
	0/6	0/6	14-day
Oxidizer/Solvent[e] (DCDMH/$CHCl_3$/t-BuOH)	6/6	6/6	30-60 mins
	6/6	6/6	24-hr
	6/6	6/6	48-hr
	6/6	6/6	72-hr
	6/6	6/6	7-day
	6/6	6/6	14-day
Wastestream[f]	6/6	6/6	30-60 mins
	6/6	6/6	24-hr
	6/6	6/6	48-hr
	6/6	6/6	72-hr
	6/6	0/6	7-day
	6/6	0/6	14-day
HD[g]	6/6	6/6	30-60 mins
	6/6	6/6	24-hr
	6/6	6/6	48-hr
	6/6	6/6	72-hr
	6/6	6/6	7-day
	0/6	0/6	14-day
HN[g]	6/6	6/6	30-60 mins
	6/6	6/6	24-hr
	6/6	6/6	48-hr
	6/6	6/6	72-hr
	6/6	6/6	7-day
	1/6	1/6	14-day

Table 12. (Continued)

Treatment Group	Incidence		
	Erythema	Edema	Time
L[g]	6/6	6/6	30-60 mins
	6/6	6/6	24-hr
	6/6	6/6	48-hr
	6/6	6/6	72-hr
	6/6	6/6	7-day
	5/6	5/6	14-day

[a] Cutaneous injury was assessed after a 4-hr occluded exposure to test article (0.5 ml). Visual assessment of the severity of erythema/edema was made per scoring system of Draize et al. (1944).
[b] Redness of the skin (severity scale 0-4).
[c] Localized swelling (severity scale 0-4).
[d] Initial observations were made 30-60 mins after "patch" removal.
[e] Decon mixture consisted of DCDMH in chloroform/t-butanol.
[f] Wastestreams consisted of oxidizer/solvent system, products and by-products of agent degradation, and residual levels (ppm) of agent.
[g] A 0.5 ml volume of agent solution [(50 ug/ml (50 ppm)] was applied. The 50 ppm concentration corresponded to the Method Quantitation Limit (MQL) of GC-MS.

The skin-injurant potential of each agent (HD, HN or L) was evaluated after agent application (0.5 ml of a 50 ppm agent solution). The 50 ppm agent level corresponded to the Method Quantitation Limit (MQL) of the GS/MS. Sulfur mustard produced grossly visible skin reaction, initially noted as slight erythema, with varying degrees of edema at 30-60 minutes after removal of the occlusive dressing. The skin response was enhanced through 24 hours typified by strongly demarcated edematous lesions (refer to Appendix M, Table M-2). Severe erythema (grade 4) and severe edema (grade 4) were hallmarks of the skin injury observed at 48 and 72 hours after patch removal. At 7 days, the edema had subsided (grade 2 (5/6)); however, the degree of erythema had not subsided. At 14 days post-exposure, recovery from the HD-induced skin lesions had essentially resolved (Scar tissue noted in 2/6 rabbits, no indication of erythema in 4/6 rabbits, and no evidence of edema in any of the treated animals). The development of the HD-induced skin lesions and healing were consistent with that described in the literature (McMaster and Hogeboom, 1945). A four-hour occluded exposure to nitrogen mustard (HN-3) also produced skin injury in rabbits (refer to Appendix M, Table M-3). Varying degrees of erythema and edema were observed at 24 hours post-exposure, moderate-to-severe erythema (grade 3) was noted at 48 and 72 hours post-exposure, and a grade 4 (severe edema) was observed at 48 and 72 hours post-treatment. By 7 days, the erythema had progressed to a grade 4 lesion (severe) in 2/6 animals whereas the edema had subsided considerably (grade 2 (2/6); grade 1 (4/6)). Assessment of the nitrogen mustard-treated animals at 14 days indicated near complete recovery to the HN-induced skin lesions (5/6 animals exhibited healing skin, 5/6 animals with no indication of edema). Exposure to lewisite also resulted in skin injury (refer to Appendix M, Table M-4). The characteristic lesion was similar to that produced by HD or HN except that the skin injury brought about

by lewisite developed more rapidly (grade 3 erythema (4/6); grade 4 erythema (2/6) and grade 4 edema (6/6) at 24 hours compared to grade 3 erythema (1/6) and grade 4 edema (5/6) animals observed 24-hr post-exposure in the HD-treated animals). Severe erythema and edema were noted at both the 48 and 72 hour observation periods. Severe erythema was persistent at 7 days concomitant with intense edema. There was little improvement in the condition of the skin noted at 14 days which contrasted to that observed in the HD or HN-treated rabbits at 14 days post-exposure. The findings reported are consistent with the view that lewisite is more damaging to the skin than sulfur and nitrogen mustards (Gates et al., 1946). Comparative skin reactions are presented in Tables 14 and 15.

3.2.1.5 DCDMH Reaction (Modified Charcoal Process Chemistry):

The wastestream from the DCDMH-mediated neutralization of CAIS (agent on charcoal) and the oxidant/solvent system [DCDMH/ $CHCl_3$/t-BuOH] were evaluated for skin effects. The amount of t-BuOH corresponded to the proportion of t-BuOH in the neutralization reaction with lewisite. The observations are presented in Appendix L, and the incidence of skin reactions (erythema/edema) are highlighted in Table 13. Exposure to oxidant/solvent system resulted in skin effects, initially noted at 30-60 mins post-exposure, as grade 3 erythema and grade 4 edema. The peak response (severe erythema and edema) occurred 48-hr post-exposure and had not subsided even after 7-days following application of test material. The 14-day observation revealed marked improvement in the condition of the skin (4/6 rabbits no erythema, 6/6 rabbits no edema). Animals exposed to "charcoal process" wastestream (composite of reactions between oxidant and HD, HN, and L) were evaluated for skin injurant action of test material. Initial observations (30-60 mins post-exposure) revealed well-defined erythema with severe edema. Skin reactions (erythema/edema) to test material were severe in nature by 48-hrs after application of test article which had not subsided at 7 days post-exposure. Observations on the 14th day post-exposure (no erythema, no edema) indicated that recovery to test material-induced skin injury had occurred. Comparative skin reactions to wastestreams and oxidant/solvent systems are highlighted in Tables 14 and 15.

3.2.1.6 HN-1 Versus HN-3 Skin Effects (Erythema/Edema):

The skin-injurant action of HN-1 and HN-3 was evaluated following a 4-hr occluded exposure to test article and the results detailed in Appendix M, Table M-5. The time course and development of injury (erythema/edema) appears to be comparable for HN-1 and HN-3. Both nitrogen mustards produced maximum degrees of erythema/edema by 48 hrs post-exposure which was still manifest at 7 days after application of test material. Indications of recovery to the skin-injurant action of test material was evident at 14 days after application. The skin irritant effects of HN-1 or HN-3 were comparable to that produced by sulfur mustard (see section 3.2.1.4). These results are consistent with the literature regarding the skin injurant action/vesicant action of nitrogen and sulfur mustards (Cope et al., 1946).

Table 13. Incidence of Skin Injury on Exposure (4-hr) to DCDMH-Neutralized Agent (HD, HN, L on charcoal), or Oxidizer/Solvent System[a]

Treatment Group	Incidence: Erythema[b]	Edema[c]	Time
Oxidant/Solvent[d]	6/6	6/6	30-60 mins[e]
	6/6	6/6	24-hr
	6/6	6/6	48-hr
	6/6	6/6	72-hr
	6/6	6/6	7-day
	2/6	0/6	14-day
Wastestream			
	6/6	6/6	30-60 mins[e]
	6/6	6/6	24-hr
	6/6	6/6	48-hr
	6/6	6/6	72-hr
	6/6	6/6	7-day
	0/6	0/6	14-day

[a] Dermal injury was evaluated after a 4-hr occluded exposure to test material (0.5 ml). Visual assessment of the degree of erythema/edema was conducted per the scoring system of Draize et al. (1944).
[b] Redness of the skin - severity scale (0-4).
[c] Localized swelling - severity scale (0-4).
[d] Decon mixture consisted of DCDMH in chloroform for reaction with HD and HN. Decon mixture consisted of DCDMH in chloroform/t-butanol for reaction with L. Mixture tested was $CHCl_3$/t-BuOH.
[e] Initial observations were conducted 30-60 mins following removal of occlusive dressing.

3.2.2 Skin Irritation Testing (24-Hr Occluded):

Rabbits that were exposed to oxidant/solvent systems and wastestreams to ascertain systemic toxicity (Limit Test/ Lethality Screen - refer to Section 2.4.3.1) were also evaluated for skin irritant effects.

3.2.2.1 Brom-55p Reaction (Initial "Blue Process" Chemistry):

The skin injurant effects of Brom-55p/sulfolane was assessed following a 4-hr occluded exposure to test material[14] (observations detailed in Appendix N, Table N-1). An intense edema (grade 4) with varying degrees of erythema (well-defined/moderate-to-severe) were observed 24-hrs post-exposure which was essentially unchanged in severity through 48 hours. At 7 days post-exposure, the degree of cutaneous damage was substantial. Since the treated animals were experiencing considerable discomfort, they were euthanized 7 days post-exposure. The skin irritant effects of the "Blue Process" wastestream, resultant from the chemical neutralization of HD with Brom-55p was determined following exposure to 1 ml/kg of wastestream. At 24-hr post-exposure, pronounced erythema (grades 3, 4) with accompanying severe (grade 4) edema were noted. The 48/72-hr observation indicated that the erythema and edema had extended well beyond the application site. The findings

[14] The Brom-55p/sulfolane exposure was limited to a 4-hr test because of the severe skin injurant action of the oxidizer/solvent mix. This action was consistent with the recommendation of the attending veterinarian in minimizing undue stress to test animals.

of the 7 and 14-day observations clearly indicated that exposure to this wastestream resulted in the production of necrotizing lesions - detailed observations are summarized in Appendix N, Table N-3. Comparison to other wastestreams and to oxidant/solvent systems is presented in Table 16.

3.2.2.2 **DCDMH Reaction (Modified Blue Process Chemistry)**:

Rabbits exposed to DCDMH/$CHCl_3$/t-BuOH solution (1 ml/kg) manifested severe edematous erythema (grade 4 erythema, edema) in 8/10 rabbits at 24 hrs post-exposure (see Appendix N, Table N-2). Cutaneous injury plateaued at 48 hrs typified by severe erythema and edema, which extended well beyond the dose site, and eschar [scab, slough (necrosed tissue)]. At 7 days post-exposure necrotic encrusted lesions were evident with little/no indication of healing skin. At 14 days post-exposure, substantial necrotic areas of skin were still manifest. Exposure to modified "Blue Process" wastestream resulted in severe edema (grade 4) in 10/10 rabbits with severe erythema (grade 4) in 7/10 animals evaluated 24 hrs post-exposure. At 48 to 72 hours post-exposure, animals exhibited moderate-to-severe (3/10) and severe (7/10) degrees of erythema concomitant with severe edema (10/10). Eschar was noted in 8/10 treated rabbits. At 7 days post-exposure recovery was evident; however, eschar was noted in 7/10 animals. Observations at 14 days post-exposure indicated that healing/recovery was on-going (e.g., edema absent) although several lesions appeared blanched and eschar was still manifest. The cutaneous injury resulting from treatment with wastestream did not appear as severe as the oxidant/solvent treated rabbits (observations are provided in Appendix N, Table N-4). Also, for comparison of skin injurant action, refer to Table 16.

3.2.2.3 **m-cpba Reaction (Initial Red Process Chemistry)**:

Occluded exposure to oxidant/solvent (m-cpba in $CHCl_3$/t-BuOH) resulted in cutaneous injury. A total of 4 rabbits were exposed to treatment (detailed observations given in Appendix O, Table O-1). The visible lesion plateaued 72 hrs post-exposure characterized by severe edema and erythema with eschar. The late lesion (7 days post-exposure) was typified by encrusted lesions with little healing evident - the 14 day condition had not changed markedly from the 7 day. Rabbits exposed to wastestream, generated from the reaction of m-cpba with CAIS containing agent (HD, HN or L) in chloroform, resulted in severe skin damage. As in the oxidant/solvent exposed animals, the visible lesion plateaued by 72 hrs post-exposure consisting of epidermal necrosis covered by fibrino-serous exudate. The skin was slow to heal as evidenced by eschar present at the 14-day observation period (refer to Appendix O, Table O-2 for observations). Table 16 highlights the skin injurant action of wastestreams and oxidant/solvent systems.

TABLE 14. Comparison of Skin Reaction (30-60 minutes Post-Exposure) after 4-Hr Occluded Exposure to CAIS Wastestreams and Oxidant/Solvent Systems and Skin Injurant Effects[a,b,c]

Treatment Group	Erythema (redness)	Edema (swelling)
Initial Blue Process Wastestream (HD/55BromP)	Moderate	None Observed
Initial Red Process Wastestream (HD/HN/L/m-CPBA/solvents)	Moderate/Severe	Moderate/Severe
Modified Blue Process Wastestream (HD/DCDMH)	Mild/Moderate	Mild/Moderate
Modified Red Process Wastestream (HD/HN/L/DCDMH/solvents)	Mild/Moderate	Severe
Red Process Oxidant/solvent (m-CPBA/solvents)	Moderate/Severe	Moderate/Severe
Red Process Oxidant/solvent (DCDMH/solvents)	Severe	Severe
Modified Charcoal Wastestream HD/HN-1/L/DCDMH/solvents/charcoal (DCDMH/solvents/charcoal)	Mild	Severe
Modified Charcoal Process Oxidant/Solvents (DCDMH/solvents/charcoal)	Moderate	Severe

[a]-Skin irritant potential evaluated using New Zealand White rabbits.
[b]-Test article (0.5 mL); 4-hr occluded exposure. Each animal served as its own control.
[c]-Observations for skin injurant action was conducted 30-60 minutes following removal of the occlusive patch.

TABLE 15. Comparison of Skin Reaction (24-Hr Post-Exposure) after 4-Hr Occluded Exposure to CAIS Wastestreams and Oxidant/Solvent Systems and Skin Injurant Effects[a,b,c]

Treatment Group	Erythema (redness)	Edema (swelling)
Initial Blue Process Wastestream (HD/55BromP)	Moderate	None Observed
Initial Red Process Wasestream (HD/HN/L/m-CPBA/solvents)	Moderate/Severe	Moderate/Severe
Modified Blue Process Wastestream (HD/DCDMH)	Mild/Moderate	Mild/Moderate
Modified Red Process Wastestream (HD/HN/L/DCDMH/solvents)	Mild/Moderate	Severe
Red Process Oxidant/solvent (m-CPBA/solvents)	Moderate/Severe	Moderate/Severe
Red Process Oxidant/solvent (DCDMH/solvents)	Severe	Severe
Modified Charcoal Process Wastestream (DCDMH/solvents/charcoal)	Mild/Moderate	Severe
Modified Charcoal Process Oxidant/solvents (DCDMH/solvents/charcoal)	Moderate	Severe

[a]Skin irritant potential evaluated using New Zealand White rabbits.
[b]-Test article (0.5 mL); 4-hr occluded exposure. Each animal served as its own control.
[c]-Observations for skin injurant action was conducted 24 hours following removal of the occlusive patch

TABLE 16. **Comparison of Skin Reaction (24-hr Post-Exposure) after 24-Hr Occluded Exposure to CAIS Wastestreams and Oxidant/Solvent Systems and Skin Injurant Effects**[a,b c]

Treatment Group	Erythema (redness)	Edema (swelling)
Initial Blue Process Wastestream (HD/55BromP)	Severe	Severe
Initial Red Process Wasestream (HD/HN/L/m-CPBA/solvents)	Severe	Severe
Modified Blue Process Wastestream (HD/DCDMH)	Moderate/Severe	Severe
Modified Red Process Wastestream (HD/HN/L/DCDMH/solvents)	Mild	Severe
Red Process Oxidant/solvent (m-CPBA/solvents)	None	Severe
Red Process Oxidant/solvent (DCDMH/solvents)	Severe	Severe
Modified Charcoal Process Wastestream (DCDMH/solvents/charcoal)	Mild/Moderate	Severe
Modified Charcoal Process Oxidant/solvents (DCDMH/solvents/charcoal)	Severe	Severe

[a]-Skin irritant potential evaluated using New Zealand White rabbits.
[b]-Test article (1.0 mL/kg); 24-hr occluded exposure. Each animal served as its own control.
[c]-Observations for skin injurant action was conducted 24 hours following application of the occlusive patch.

3.2.2.4 **DCDMH Reaction (Modified Red Process Chemistry)**:

A 24-hr occluded exposure to wastestream [components of the neutralization reaction between DCDMH and agent (HD, HN, L) in $CHCl_3$/BuOH] resulted in severe cutaneous injury. Observations for each animal for each observation period are presented in Appendix O, Table O-3. Severe erythema and edema were manifest 24-hrs post-exposure. By 48 to 72 hours, several of the lesions appeared blanched, epidermal lesions covered by eschar, were present. At 7 days post-exposure, the degree of edema had subsided (grade 2 or 3) indicating healing; however, eschar was still evident in 10/10 rabbits. The 14-day observations indicated nil to slight edema with eschar seen in all treated animals. For comparison of the skin injurant action to that of other wastestreams and oxidant/solvent systems refer to Table 16.

3.2.2.5 **DCDMH Reaction (Modified Charcoal Process Chemistry)**:

Animals were assessed for skin irritation after a 24-hr occluded exposure to the oxidant/solvent system of the "Charcoal Process" chemistry. Treatment resulted in severe skin injury [erythema (4); edema (4)] at 24-hrs post-exposure. The severity of the skin response persisted through the 7th day post-exposure; and indications of recovery were noted on the 14-day post-exposure - for details, refer to Appendix P, Table P-1.

Ten rabbits were evaluated for skin irritant effects following a 24-hr occluded exposure to "Charcoal Process" wastestream. Treatment resulted in severe cutaneous injury. Observations for each animal for each observation period are tabulated in Appendix P, Table P-2. A varied erythema response (grades 2-4), with severe edema (grade 4), was noted at 24-hrs post-exposure. The erythema response had plateaued by 72 hrs post-exposure. At the 7-day observation, the level of skin injury had not subsided. The 14-day observations indicated the absence of edema; however, the erythema component of the skin reaction to test article was still categorized as severe. Comparison to skin irritant effects of the other wastestreams and oxidant/solvent systems is highlighted in Table 16.

3.2.3 **Limit Test/Lethality Screen**.

The acute percutaneous toxicity of the various oxidant/solvent systems and of neutralized CAIS was assessed after a 24-hr occluded exposure to test article at a pre-determined dose of 1 ml/kg (≈1 g/kg). Animals were monitored for toxic signs, consistent with the target organ effects of oxidant (m-cpba, Brom-55p or DCDMH), solvent ($CHCl_3$, t-BuOH or sulfolane), agent (HD, HN, L), and agent degradation products (e.g. HD sulfone, HD sulfoxide, divinyl sulfone, HN oxide, L oxide). Toxic effects, characteristic of the afore-mentioned compounds, was previously highlighted (refer to Table 5).

Results from the dermal exposure to the various oxidant/solvent systems may be summarized as follows: (1) Rabbits exposed to Brom-55p/sulfolane (oxidant/solvent system for the

initial "Blue Process" Chemistry), at a dose of 1 ml/kg, were free of overt signs of toxicity. Nil to minimal toxic effects were anticipated on treatment with "test article" since the amount of sulfolane present in the mixture represented a quantity considerably less than the amount reported for the rabbit dermal LD_{50} (3.2 g/kg), and the quantity of Brom-55p in solution represented a fraction of the rabbit dermal LD_{50} (> 20 g/kg). (2) Rabbits exposed to a mixture of m-cpba/$CHCl_3$/t-BuOH (oxidant/solvent system for the initial "Red Process" Chemistry), at a dose level of 1 ml/kg, did not manifest toxic signs of systemic poisoning. The absence of toxic signs was not unexpected since the quantity of m-cpba/$CHCl_3$/t-BuOH solution applied to the animals represented an amount considered a fraction of the reported toxic dose (LD_{50}/LDLo/TDLo) for these compounds [m-cpba: mouse dermal TDLo (21 g/kg); $CHCl_3$: rabbit dermal LD_{50} (> 20 g/kg); t-BuOH: rabbit oral LDLo (4.5 g/kg)]. (3) Rabbits exposed to test article [(0.555 M DCDMH in $CHCl_3$/t-BuOH/H_2O) - the oxidant/solvent system for the modified "Blue", "Red", and "Charcoal" process chemistries], at a dose level of 1 ml/kg, did not exhibit toxic signs attributable to the modified "Blue Process" chemistry oxidant/solvent system components (DCDMH/$CHCl_3$ with or without t-BuOH) or toxic responses resultant from additive/synergistic effects of these compounds.

Dermal exposure to wastestreams generated from the "Blue Process" chemistries [(1) Brom-55p mediated reaction in sulfolane and (2) DCDMH-mediated reaction in $CHCl_3$/t-BuOH/H_2O], utilized for the chemical neutralization of CAIS containing HD, resulted in no overt signs of systemic toxicity (e.g., narcosis, ataxia, dyspnea, respiratory depression, CNS depression, convulsions) that may be induced by oxidant or solvent or a combination of both. Toxic signs (e.g., lacrimation, salivation, diarrhea, vomiting, cachexia, hyperpnea, tremors, convulsion) indicative of systemic intoxication to sulfur mustard and/or HD degradation products (e.g., HD sulfone, HD sulfoxide and/or chlorinated derivatives) were not observed in animals treated with wastestream resultant from reaction of HD and Brom-55p. Evidence of systemic intoxication; however, was noted in rabbits exposed to wastestream generated from the reaction of DCDMH with HD. Within several hours after application of "test article", toxic signs (lacrimation and salivation - physiologic endpoints indicative of HD and/or HD-degradation product toxicity) were noted in 4/10 wastestream-treated rabbits. No lethality was noted on treatment of animals with either "Blue Process" wastestream - see Table 17.

Initial assessment of the "Red Process" (m-cpba-mediated) wastestream in rabbits demonstrated that the exposure to "test article" at a dose of 1 ml/kg resulted in systemic intoxication. Tremors were noted at 72 hrs post-exposure in 1/4 treated rabbits, and more importantly 3/4 deaths (2, 4, and 6-days post-exposure). The cause-and-effect relationship between exposure to wastestream and the mortality observed in 3/4 treated animals may have been straight forward were it not for the post-exposure treatment of these animals

with an analgesic (Buprenex®).[15] Buprenex® is known to cause gastrointestinal effects and anorexia (Liles and Flecknell, 1992) which may have aggravated the already compromised condition of the animals following exposure to wastestream. A follow-on study was conducted in 10 rabbits in which the animals were dosed with the wastestream from the m-cpba reaction without post-exposure treatment with analgesic. Within this group, overt signs of toxicity (tremors, mild convulsions, salivation) were observed in only one animal on the first day post-exposure - all other animals were free of overt toxic effects. The animal manifesting neurologic signs eventually succumbed to treatment at approximately 48 hrs post-exposure. Due to the mortalities observed at the 1.0 ml/kg dose of m-cpba wastestream, a group of 10 rabbits were administered 0.5 ml/kg of test material. Toxic signs and lethalities did not result on administration of test material at this dose level. It is conceivable that the overt toxicity expressed in two of the m-cpba wastestream (1 ml/kg) treated animals may have been due in large part to the mustard degradation product, HD sulfone, which was detected in high concentration (area%: 20.9) and perhaps in some measure to total agent (HD, HN, L) content (ca 450 ppm).

Rabbits treated with wastestream from the DCDMH-mediated reaction with HD, HN, or L in chloroform, were free of toxic effects characteristic of agent, agent degradation products and/or combination thereof. The amount of oxidant and solvent in the wastestream did not elicit toxic effects characteristic of DCDMH, $CHCl_3$, or t-BuOH or a spectrum of responses resultant from interactions (pharmacologic/toxicologic) between mixture constituents.

Dermal exposure to the wastestreams from the "Charcoal Process" chemistry (DCDMH/$CHCl_3$ used in HD and HN neutralization reactions; DCDMH/$CHCl_3$/t-BuOH used in L neutralization reaction) resulted in no overt toxicologic effects attributable to agent, agent degradation products or to a combination of toxicants despite the very complex chemical composition of the "Charcoal Process" wastestream. Oxidant and/or solvent-induced toxicity was not manifest in charcoal wastestream-exposed rabbits. A synopsis of the systemic effects observed in rabbits exposed to the various wastestreams is presented in Table 17.

[15] **Buprenorphine hydrochloride (Buprenex®, Norwich Eaton Pharmaceuticals, Norwich, NJ) was administered via the i.m. route (0.025 mg/kg, 2x/day).**

Table 17. Systemic Effects Summary: Rabbits Dermally Exposed to Various CAIS Wastestreams[a]

Wastestreams	Number of Animals	Systemic Effects[b]
Initial Blue Process (Brom-55p mediated)	(2)[c]	None Observed; lethality (none)
Modified Blue Process (DCDMH-mediated)	(10)	Lacrimation/salivation (4/10); lethality (none)
Initial Red Process (m-cpba mediated)	(14)[d]	Tremors (1/4); lethality (3/4)[e] Tremors, mild convulsions, salivation (1/10); lethality (1/10)[f]
	(10)[g]	None observed; lethality (none)
Modified Red Process (DCDMH-mediated)	(20)	None observed; lethality (none)
Modified Charcoal Process[h] (DCDMH-mediated)	(10)	None observed; lethality (none)

- a **24-hr occluded exposure; "test article" at a dose of 1 ml/kg (initial "Red Process" at two dose levels); 14-day observation period.**
- b **Toxic signs consistent with target organ effects of agent(s)/agent degradation products/oxidant/solvent(s).**
- c **Small number of animals utilized due to compliance with veterinary concerns regarding testing with this material.**
- d **Animals dosed with 1.0 ml/kg of test material.**
- e **Initial group of four rabbits treated with m-cpba wastestream were dosed with an analgesic (Buprenex®) during the post-exposure period. Within this group, one of the three mortalities occurred within 24-hrs (prior to Buprenex® treatment) suggestive of systemic intoxication due to agent and/or agent degradation products or a combination thereof.**
- f **This group of animals treated with "Red Process" wastestream were not dosed with (Buprenex®) during the post-exposure period.**
- g **Animals dosed with 0.5 ml/kg of test material**
- h **Modified process used CHCl3/t-BuOH as co-solvent for neutralization of L; $CHCl_3$ as solvent for neutralization of HD and HN.**

3.3 Data Analysis Results.

Statistical analyses of the skin irritation scores based on readings at 30-60 mins after patch removal following a 4-hr occluded exposure to 0.5 ml of "test article" revealed the following: (1) No significant difference in skin response to initial "Blue Process" oxidant/solvent system and wastestream; (2) A significantly more severe skin reaction on exposure to modified "Blue Process" oxidant/solvent system compared to the response observed after treatment with modified "Blue Process" wastestream; (3) Exposure to oxidizer/solvent system of the modified "Red Process" chemistry resulted in a significantly greater erythema response compared to that observed in animals treated with modified "Red Process" wastestream; and (4) The skin reaction (for erythema) was significantly more severe on treatment with oxidizer/solvent system

than with wastestream ("Charcoal Process" chemistry).

Statistical analyses of the skin irritation scores (24-hr) following a 4-hr contact to 0.5 ml of "test article" indicated the following: (1) No significant differences in skin response between initial "Blue Process" oxidant/solvent system and wastestream; (2) A more severe (statistically significant) skin response after treatment with modified "Blue Process" chemistry oxidizer/solvent system compared to wastestream-induced skin reaction; (3) A more severe erythema response in the oxidizer/solvent system (modified "Red Process") treated group versus the wastestream-treated group; and (4) No significant difference in the skin reactions to the wastestream and oxidizer/solvent system of the "Charcoal Process" chemistry.

Statistical analyses of the skin response based on observations at the conclusion of the 24-hr occluded exposure to 1.0 ml/kg of "test article" revealed the following: (1) No significant difference for Draize indices (erythema/edema) scores following treatment with either solvent/oxidant system or wastestream of the modified "Blue Process" chemistry; (2) No significant difference in skin reaction between oxidant/solvent system and wastestream (initial "Red Process" chemistry); and (3) A greater (significant) severity in the skin response to the oxidizer/solvent system compared to wastestream ("Charcoal Process" chemistry).

Statistical analyses of the skin response at 7 days post-exposure after treatment with "test article" (4-hr occluded exposure, 0.5 ml of test material) revealed the following: (1) The initial "Blue Process" chemistry (oxidant/solvent system and wastestream) treatment resulted in no significant differences in skin response; (2) The oxidizer/solvent system (modified "Blue Process" chemistry) resulted in a significantly more severe edema response than obtained on treatment with wastestream; (3) Skin reactions were significantly more severe for the oxidant/solvent system treated animals than those obtained on treatment with the wastestream (modified "Red Process" chemistry); and (4) No significant differences were noted between oxidant/solvent system and wastestream treated animals of the "Charcoal Process" chemistry in terms of Draize scores.

Statistical evaluation of the data support the null hypothesis: that treatment with wastestream resulted in skin injury (erythema/edema) equal to or less in severity to that induced by oxidant/solvent system treatment.

4. DISCUSSION

The intent of the process chemistries was to develop chemical neutralization reactions that achieved destruction of CAIS agents with minimal toxic hazards associated with the process chemistries and resultant products of neutralization. The above objectives represented a formidable challenge since the chemical

neutralization of the agents, particularly involving sulfur mustard, and to a lesser degree lewisite and nitrogen mustard, can result in the formation of reaction products/by-products having vesicant action and/or a high degree of systemic toxicity. The degradation of agents involves complex chemical reactions which is certainly the case for sulfur mustard. HD degradation is complicated by the presence of sulfur and chlorine in the HD molecule which in some cases facilitates and in others impedes the chemical degradation of HD. Over the years, the neutralization chemistry of HD has focused on the reaction of sulfur mustard as the sulfide. Feasible methods for the destruction of HD have included oxidation and chlorination and oxidation for nitrogen mustard and lewisite. The toxicity of degradation products resultant from the chemical neutralization of HD, HN, or L is of concern to the toxicology, health, and regulatory communities. Discussion initially focuses on the effectiveness of the neutralization processes in reducing the high systemic toxicity of the agents followed by discussion pertaining to the outcomes of the skin toxicity tests, as well as, addressing the topic of agent and degradation product vesicancy. It must be kept in mind that the current studies were undertaken to assess the systemic toxicity and skin irritant potential and not to elucidate the vesicant properties of neutralized CAIS. The discussion also reflects the interdynamics between process chemistries development and toxicologic assessment - the aim of which is to provide chemistry methodologies capable of producing marked reduction in vesicant activity and minimal health risks.

It has long since been known, that aside from their localized action (e.g., irritation, vesication, ocular effects, and respiratory effects), blistering agents have appreciable systemic toxicity. Dermal LD_{50} values [HD (~40 mg/kg); HN (~15 mg/kg); and L (~5 mg/kg)] for the blistering agents attest to the highly toxic nature of these compounds. Current methods for demilitarizing CAIS is still based largely on chemical neutralization via oxidizing materials although the use of DCDMH as oxidant does provide an adjunct degradation pathway via the chlorination of HD.[16] The oxidation of sulfur mustard, as pointed out by Franke (1967), represents one of the most important decontamination reactions for HD. The oxidation of sulfur mustard via various oxidizers (e.g., hydrogen peroxide, hypochloric acid and its salts, potassium permanganate, nitric acid, m-cpba, DCDMH, Brom-55p, etc.) yields various compounds whose composition depends on the nature of the oxidant used and the specific reaction conditions. Most easily formed is HD sulfoxide which on oxidation yields HD sulfone - both represent major oxidation products of sulfur mustard. Rigorous oxidation of HD leads to the formation of β-chlorethane sulfonic acid, and the complete destruction of HD can occur under certain conditions.

[16] HD is easily destroyed by all chlorinating agents (aqueous or anhydrous medium). Under appropriate conditions, the chlorination of HD can proceed to form various polychlorides. In the presence of water, chlorination of HD is altered resulting in the formation as sulfoxides (Aleksandrov, 1969).

Table 18. Acute Toxicity of Agents Versus Various Degradation Products[a]

Compounds	Systemic Toxicity (LD50 mg/kg)[b] s.c.	i.p.
Agents		
HD	(26)[c]	(-)[b]
HN-1	(1-2)	(1-1.8)
HN-3	(2-10)	(-)
L	(≈1)[d]	(>2)[e]
Degradation Products[f]		
HD sulfoxide	(>125)[g]	(100)
Divinyl sulfoxide	(>150)[h]	(-)
HD sulfone	(35)[i]	(-)
Divinyl sulfone	(16)	(-)
α,α,β,β,β,β hexachlorodiethyl sulfide	(>350)[j]	(-)
β-chloroethyl α,β-diclorovinyl sulfide	(>800)[k]	(-)
β-chloroethyl, α,β,β-trichlorovinyl sulfide	(>1200)[l]	(-)
HN-1 oxide	(-)	(50-100)
HN-3 oxide	(-)	(2-5)
L oxide	(≈ 3)[m]	(-)
2-chlorovinylarsonic acid	(-)	(>500 mg/kg)[n]
2-chlorovinylarsonous acid	(-)	(-)

a Data obtained in mice unless otherwise stated; route of exposure subcutaneous (s.c.) and/or intraperitoneal (i.p.).
b Precise LD_{50} values not always available. In some instances, a range is given [e.g., HN-1, HN-3 - Range based on the form of HN (HN as the free base or as the hydrochloride)]. Also, when an LD_{50} dose is not available, the minimal lethal dose (MLD) is given. In some instances, acute toxicity data for the above routes of exposure are lacking [indicated by (-)].
c Minimal lethal doses of 90 and 125 mg/kg (mouse, s.c.) have also been reported.
d Value is for rat s.c. LD_{50} dose.
e Value is based on LDLo (MLD) of 2 mg/kg (guinea pig).
f Generally, the degradation products are those most-commonly reported for blistering agents that have been studied for biologic effect. It is not the intention of the authors to provide an exhaustive listing of degradation products.
g Based on a MLD of 125 mg/kg (mouse, s.c.).
h Based on a MLD of 150 mg/kg (mouse, s.c.).
I Previous toxicity data reported an MLD of 105 mg/kg (mouse, s.c.).
j Based on a MLD of 350 mg/kg (mouse, s.c.).
k Based on a MLD 800 mg/kg (mouse, s.c.).
l Based on a MLD (>1200 mg/kg, mouse s.c.).
m Value approximated based on lethality screen data (mouse, s.c.) as follows: 10 mg/kg (5/5); 5 mg/kg (5/5); 2 mg/kg (0/5).
n Value approximated based on lethality screen data (mouse, i.p.) as follows: 1000 mg/kg (10/10); 500 mg/kg (0/10).

The oxidation of HD not only alters the skin-damaging properties of HD but the systemic toxicity of sulfur mustard as well. The oxidation of HD is of great interest since sulfoxide formation, on chemical neutralization of HD, can be considered a "detoxification". The "detoxification" of HD via oxidation to the sulfoxide was demonstrated in the 1940's. In contrast, the formation of mustard sulfone, a product of further oxidation, can contribute to an enhanced systemic toxicity and vesicant potential of the product solution/mixture. It is generally accepted that HD sulfoxide is less acutely toxic than HD [LD_{50} of HD sulfoxide (>125 mg/kg, s.c.); LD_{50} of HD (26 mg/kg, s.c.)] - refer to Table 18. On the other hand, HD sulfone, having the **$S(O)_2$** functional group, is highly poisonous and comparable in toxicity to HD (HD sulfone: LD_{50} = 35 mg/kg, s.c.) - refer to Table 18. Research conducted since Philips' review (Philips, 1950) on sulfur mustard pharmacology/toxicology demonstrated that HD sulfone is a highly toxic vesicant. In regard to HD sulfone toxicology, the following has been stated: "Of these oxidation products, the poisonous one is sulfone, where toxicity is commensurate with the toxicity of yperite itself" (Aleksandrov, 1969). Finally, one needs to discuss briefly the aspect of vinyl containing derivatives which may form on chemical neutralization of agent - another area of concern relevant to the toxicity characteristics of the product solutions. It is generally regarded that compounds containing the vinyl group **(C=C)** are more reactive and associated with a higher degree of toxicity than the corresponding saturated compounds. For instance, with regard to aliphatic hydrocarbons, alkenes are more reactive than alkanes - a characteristic responsible for their higher toxicity (Sandmeyer, 1981). Further, diunsaturation (multiple double bonds), in general, also increases toxicity. Altered toxicity, owing to the presence of a vinyl group, is also relevant to sulfur mustard toxicity. Formation of reactive vinyl groups (via elimination of hydrogen and chlorine) can occur involving both oxidized and non-oxidized derivatives of HD. The presence of the double bond imparts a higher degree of toxicity. Smith (1943c) has shown that divinyl sulfone was more toxic than HD sulfone on parenteral administration. Anslow et al. (1948), on examining the toxicity of HD and its various derivatives, reported the enhanced toxicity of vinyl containing analogs (e.g., HD sulfone: s.c. $LD_{50} \approx 35$ mg/kg; divinyl sulfone: s.c. $LD_{50} \approx 16$ mg/kg). Certainly, based on the known toxicity characteristics of mustard sulfone, mustard sulfoxide, and their vinyl derivatives; it is crucial that the process chemistries developed for the destruction of CAIS employ oxidants that minimize the formation of HD sulfone and HD analogs having comparable biological activity (systemic toxicity and vesicancy) to that of HD.

The success in developing a process chemistry that achieves reduced health risks was most evident in studies related to the "Red Process" chemistry wastestreams. Firstly, it must be stated that both

Table 19. Vesication Potential of Various Analogs/Derivatives of Sulfur Mustard

Analogs/Derivatives (Saturated and Unsaturated)	Vesicant Activity	References[a]
OXIDIZED DERIVATIVES		
Mustard Sulfone [sulfone, bis(2-chloroethyl)]	(POS)	Marshall & Williams (1921); Young et al. (1944)
Sulfone, 2-chloroethyl vinyl	(POS)	Young et al. (1944)
Divinyl Sulfone	(POS)	Young et al. (1944); Thomson et al. (1945)
Mustard Sulfoxide [sulfoxide, bis(2-chloroethyl)]	(NEG)	Marshall & Williams (1921); Lawson & Dawson (1927); Fuson et al. (1943); Bergmann et al. (1945)
Divinyl Sulfoxide	(NEG)	Young et al. (1944); Thomson et al. (1945); Bergmann et al. (1945)
β-chloroethyl vinyl sulfoxide	(NEG)	Young et al. (1944)
α,β,β'-trichlorodiethyl sulfoxide	(NEG)	Young et al. (1944)
CHLORINATED DERIVATIVES		
bis(α-chloroethyl) sulfide	(NEG)	Peters and Walker (1923); Baldwin et al. (1924); Kirner (1928); Dawson & Wardell (1930).
α,β,β'-trichlorodiethyl sulfide	(NEG)	Mann & Pope (1922); Lawson & Dawson (1927).
α,β,β,β' tetrachlorodiethyl sulfide	(NEG)	Mann & Pope (1922); Lawson & Dawson (1927)
α,α',β,β' tetrachlorodiethyl sulfide	(NEG)	Lawson and Dawson (1927).
α,α,β,β,β,β' hexachlorodiethyl sulfide	(NEG)	Mann & Pope (1922); Lawson & Dawson (1926); Dawson & Wardell (1930).
β-chloroethyl α,β dichlorovinyl sulfide	(NEG)	Lawson & Dawson (1926); Kirner (1928); Dawson & Wardell (1930)
β-chloroethyl α,β,β' trichlorovinyl sulfide	(NEG)	Lawson & Dawson (1926); Kirner (1928); Dawson & Wardell (1930).
β-chloroethyl chlorovinyl sulfide (α and β isomers)	(POS)	Lawson & Dawson (1926); Dawson & Wardell (1930); Fuson et al. (1943).

[a] **Citations are primary and/or secondary.**

"Red Process" chemistries (initial and modified) achieved a major reduction in the acute toxicity potential of CAIS, containing agent in chloroform, since the animals treated with wastestreams (composite of HD, HN, and L reactions with oxidant) from the neutralization of agents were notably free of toxic signs. As indicated previously, the fairly high agent content (ca 450 ppm, refer to Table 8) may have contributed to the overt toxic effects. An obscure report (Wilson et al., 1943) detailed the effects in rats of low levels (ca 100 ppm) of HN-3 in aqueous solution given ad libitum or via gavage (single dose) at concentrations up to approximately 350 ppm. The authors reported generic toxic effects (i.e., loss of appetite). The investigators conceded that the toxicity of the aqueous solutions containing HN-3

was not readily resolvable due in part to the instability of test solutions. Nevertheless, this early study pointed to the possibility of toxic effect(s) - albeit minor - as a consequence of low-level exposure to agent material.

Concern regarding the toxicity characteristics of the wastestreams must also focus on the degradation products resultant from the chemical neutralization of agent. Analyses of the wastestreams via gas chromatography/mass spectrometry (GC/MS) indicated the presence of HD sulfone, HD sulfoxide, and other oxidation products (saturated and unsaturated). The relationship between HD sulfone and HD sulfoxide content (and perhaps that of other constituents) and systemic toxicity, and the impact on process chemistry methodology was most apparent on study of the "Red Process" wastestreams. The initial "Red Process" chemistry used m-cpba (stronger oxidant than DCDMH) whereas the modified "Red Process" chemistry used DCDMH. Both treatment reagents resulted in the oxidation of HD to the sulfoxide and sulfone analogs. However, there was a marked reduction in HD sulfone content in the product solution from the DCDMH reaction. (TIC area percent: 0.5%) compared to a very high HD sulfone content (TIC area percent: 20.9%) in the m-cpba mediated neutralization of HD. The analytical results were consistent with the postulate that DCDMH selectively oxidizes HD to the sulfoxide compared with the more rigorous oxidation of HD to the sulfone via m-cpba. The results of the lethality screen are consistent with the analytical data regarding HD sulfone content. Tremors, mild convulsions, and salivation - characteristic of exposure not only to β-chloroethyl vesicants but to the oxidized derivatives as well - was noted in 1/10 animals exposed to wastestream generated from the neutralization of CAIS (HD, HN, or L in chloroform) with m-cpba. A single lethality (animal manifesting tremors and other toxic signs which died ≈ 72 hrs post-exposure) was also noted in this treatment group - refer to Table 17. These findings contrasted sharply with results following the treatment of animals with DCDMH-generated wastestream where overt toxic signs were absent and lethality had not occurred. Although the toxicity data on the "Red Process" wastestreams provide strong support for postulating HD sulfone as the moiety most likely to have contributed to the systemic intoxication seen in the one animal exhibiting overt toxicity, one cannot exclude other wastestream components (most likely other degradation products) as contributing to the overall toxicity observed. The wastestream tested is a complex mixture (as are all wastestreams) containing residual agent (HD, HN, and L) as well as degradation products/by-products. The gross toxicity observed may also have been due in part to one or more interactive effects (e.g., additive, synergistic, potentiation) involving other components present. The constituents singly or in combination may have contributed to the total systemic toxicity - it must be kept in mind, that even HD sulfoxide, long regarded as less toxic than either HD or its highly toxic analogs (e.g., HD sulfone, divinyl sulfone), is not without toxic effect(s) [Voegtlin et al. reported toxic manifestations on exposure to HD sulfoxide as cited by Anslow and Houck, 1946].

The findings related to the "Blue Process" chemistries and

wastestreams toxicity assessment merits discussion. Preliminary analytical results indicated a dramatically lower sulfides content in the wastestream from the DCDMH-mediated neutralization reaction compared to the high sulfides content in the Brom-55p wastestream. Also, in the same preliminary investigations, the DCDMH-mediated neutralization resulted in lower residual HD. These results served as an impetus to explore further the DCDMH process chemistry. The "Blue Process" studies demonstrated that attainment of all the desired objectives (e.g., process simplicity, marked reduction in agent characteristic, reduced toxicity characteristics) may not be fully realized. The conditions of the DCDMH-mediated neutralization, which resulted in lowered residual HD levels (i.e., ≤ 50 ppm), had, unfortunately, the following additional outcomes: (1) production of a more complex mixture, (2) favored the formation of numerous vinyl containing degradation products [total vinyl containing moieties (area %: 76.1%)], and (3) increased the amount of unknowns (7.7%) in the mixture. Using mortality as the sole index of toxicity, one may postulate that both "Blue Process" chemistries effectively degraded sulfur mustard to less toxic products. The collective data, particularly from the testing of Brom-55p wastestream, would suggest that the chemical neutralization of HD resulted in a "detoxification" of agent. In comparison, the degree of "detoxification" via DCDMH was not as great, evidenced by the occurrence of sublethal toxic signs (lacrimation, salivation) in the DCDMH wastestream-treated rabbits. The observed toxicity is consistent with the high level of vinyl-containing compounds, which have greater toxicity potential than their corresponding saturated analogs. Also, since the DCDMH mixture contained more components (nearly double) than that of the Brom-55p wastestreams; one must not exclude the likelihood that additive/synergistic effects, involving wastestream components, may have also contributed to the total systemic effects seen. Finally, regarding agent content, it can be stated that the residual level of HD detected in both wastestreams would not have contributed to the overall systemic toxicity observed since ppm concentrations detected (i.e., ≤100 ppm) are below amounts associated with overt signs of toxicity.

The final segment of the discussion addresses the issue of agent and degradation product vesicancy potential and is germane to this report, since part of the current efforts entailed an assessment of the process chemistries in relation to wastestream analysis and existent toxicity data on the vesicancy potential of agent and/or agent degradation products/by-products. The combined chemistry and toxicologic data was utilized to arrive at an initial evaluation of a process chemistry to effectuate reduction of agent characteristics. It is imperative to point out that the above undertaking is by no means a replacement for actual vesicancy testing of the wastestreams, which are on-going at the time of this writing. The current process chemistries utilized various oxidizing agents which in the case of m-cpba and DCDMH-mediated reactions can result in the generation of highly chlorinated derivatives of sulfur mustard. Component analysis of the wastestreams, particularly for the charcoal process, indicated the presence of several compounds with high chlorine content. Trichloro mustard (1,2,2-trichloro diethyl sulfide) was a major constituent in the wastestreams from the initial charcoal process

chemistry. The concern that the aforementioned derivative, as well as, other chlorinated analogs may possess vesicant activity prompted an extensive review of the toxicology literature on the dermal toxicity/vesication properties of the chlorination products of sulfur mustard - information which is summarized in Table 19.

For purposes of this report, discussion on the relationship between chemical structure and vesication is limited to the thioether molecule. Degradation product(s) of the nitrogen mustards have not been implicated as having vesicant potential although this area of research needs to be explored. The principal degradation product of lewisite, namely, L oxide is a potent vesicant. The reader is referred to several papers/reviews on the subject of mustard vesication and toxicology (Bouder, 1940; Anslow and Houck, 1946; Philips, 1950; Aleksandrov, 1969; Franke, 1967; and Henry, 1991), as well as reviews covering the systemic toxicity and pathology of nitrogen mustards (Anslow and Houck, 1946; Renshaw, 1946; Cope et al., 1946; and Graef et al., 1948). The subject of lewisite toxicology and pathology has also been amply covered (Wardell, 1941; Gates et al., 1946; and Goldman and Dacre, 1989).

The vesicant potential of sulfur mustard derivatives (oxidation and chlorination products) has been investigated since the 1920's to modern times. Research has indicated that the strongest vesicant action is exerted by β-halogenated sulfides. The position and degree of chlorination influences the vesicant potential of the thioether molecule. With respect to the site of chlorination, Kirner (1928) and Dawson and Wardell (1930) concluded that compounds having the chlorine atom in the beta position were considerably more vesicant that those having chlorine in the alpha or gamma position. The degree of chlorination also influences the vesicant activity of the sulfide molecule and hence the early use of chlorination to degrade HD. Monosubstitution analogs of HD, regardless of position, are less effective vesicants than HD. As previously stated, the introduction of halogen atoms results in decreased toxicity and markedly diminished vesicant action. Research in the 1920s (Mann and Pope, 1922; Peters and Walker, 1923; and Lawson and Dawson, 1927)-summarized by Bouder (1940)-indicated that the higher chlorinated derivatives (e.g., tri-, tetra-, and hexachloro derivatives) of HD (saturated or unsaturated) were non-vesicant. Acute toxicity profiles and summary of the vesicant potential of various chlorinated analogs of sulfur mustard are given in Tables 6 and 19. The demilitarization of CAIS as stated is based on chemical neutralization via oxidizing materials which not only alters the systemic toxicity of HD (as discussed) but the skin damaging properties (irritation, vesication). Fuson et al. (1943) on review of the vesicant activity of sulfur compounds concluded that compounds containing the **S(0)** group were non-vesicant. Mustard sulfone, containing the **$S(0)_2$** functional group, is a known vesicant (vesicancy potential 1/7 to 1/5 of HD (Bergmann et al., 1945). The formation of HD sulfone can contribute to an enhanced systemic toxicity (as per discussions pertinent to the "Red Process" chemistries) and vesicant potential of the product solution/mixture (wastestream). The vesicant potential of the wastestreams are under evaluation and will be reported separately.

5. CONCLUSIONS AND RECOMMENDATIONS

Based on the findings of these studies, coupled with existent information in the literature, the following conclusions can be made:

- Data indicate that a "Packing Group I" poison (sulfur mustard) was destroyed by reaction with DCDMH or Brom-55p and converted to less toxic materials "Packing Group III" poison according to biological criterion set forth in 49 CFR (Department of Transportation-Research and Special Programs Administration).

- Data indicate that sulfur mustard (HD) nitrogen mustard (HN-1 or HN-3) and Lewisite (L) ("Packing Group I") poisons were destroyed by reaction with DCDMH and converted to less toxic materials "Packing Group III" poison according criterion set forth in 49 CFR (Department of Transportation- Research and Special Programs Administration).

- Pertaining to the "Blue Process" chemistry, data suggest that the DCDMH-mediated reaction produced product solutions with a greater degree of toxicity than the wastestreams resultant from the Brom-55p mediated reaction.

- Pertaining to the "Red Process" chemistry, data (sublethal effects) suggests that the m-CPBA-mediated reaction produced product solutions with a greater degree of toxicity than that resultant from the DCDMH-mediated reactions. The modified "Red Process" chemistry (DCDMH-mediated) was highly effective in reducing toxic moieties such as HD sulfone which is a known vesicant.

- The modified "Blue Process" chemistry (DCDMH as oxidant) resulted in a complex mixture, in the formation of numerous vinyl-containing degradation products, and an increased amount of unknowns which could potentially result in enhanced toxicity. The presence of numerous vinyl moieties, some possessing the required structure/activity for vesication, may present an added concern relevant to vesication potential.

- The "Charcoal Process" chemistry, although resulting in the detoxification of the agents (HD, HN, and L), generated a product solution that was extremely complex with many unknowns.

- Products/by-products can be produced in all neutralization reactions that retain considerable toxicity and/or potential vesicant action.

- The oxidizer/solvent systems alone, when tested for skin irritant action, gave responses equivalent to or greater than those seen with the wastestreams. These results support the determination that the wastestreams can be transported per shipping designations established for the oxidants or solvents used in the RRS process chemistries.

Relevant to the findings of these studies, the following recommendations are offered:

- It is recommended that the analytical techniques used for the characterization of wastestreams be further refined to reduce the number of unknowns reported in the wastestream analyses.

- It is recommended that dermal toxicity tests be conducted on "field"/"site" obtained CAIS.

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APPENDIX A

TOXICOLOGIC PROPERTIES OF THE BLISTERING AGENTS HD, HN, L

Toxicologic Profile of Sulfur Mustard (HD)

Toxicologic Profile of Nitrogen Mustard (HN)

Toxicologic Profile of Lewisite (L)

TOXICITY PROFILE: SULFUR MUSTARD (HD)

The following represents a synopsis on the toxicologic/ pharmacologic characteristics of sulfur mustard. The data base was assessed via manual and on-line searches.

Chemical and Physical Properties

CAS Registry No.: 505-60-2

Chemical Name and Synonyms: Sulfide, bis(2-chloroethyl)
2,2-dichloroethyl sulfide
bis-(2-chloroethyl) sulfide

Trade Name and Synonyms: HD, HS
Sulfur Mustard
Yperite

Mol. Formula: $C_4H_8Cl_2S$

Structural Formula: $ClCH_2CH_2SCH_2CH_2Cl$

Mol. wt: 159

Sulfur mustard is an oily, colorless to amber liquid with a garlic odor. HD is slightly soluble in water (<1%); freely soluble in alcohol, acetone, carbon tetrachloride, fats and oils. Sulfur mustard undergoes hydrolysis (only if dissolved) - hydrolysis products are thioglycol and hydrogen chloride. Sulfur mustard is also oxidized by various oxidants (e.g., hydrogen peroxide, hypochloric acid and its salts, potassium permanganate, hydantoins) to yield compounds whose composition depends on the nature of the oxidant used and specific reaction conditions.

Health Hazards Data/Health Effects Data

Target Organs:

Skin, eyes, lungs, gastrointestinal tract, hematopoietic (blood forming organs), central nervous system (CNS).

Acute Toxicity:

Sulfur mustard is both a locally acting and systemic toxicant. It is insidious in action (immediate symptoms to not accompany exposure) and can damage any tissue which comes in contact with it. Sulfur mustard injury on dermal or inhalation

exposure is chiefly characterized by localized action involving skin, lungs and eyes - severe exposure can result in significant ocular damage and severe lung edema. In humans, systemic intoxication will usually occur in conjunction with extensive local injury. In animals, systemic effects can be produced on parenteral administration in the absence of local HD effects. Sulfur mustard can result in appreciable systemic toxicity involving on array of organ systems, and there is a relatively asymptomatic latent period (several hrs to one day). In man, early symptoms of exposure include inflammation of the eyes, nose, throat and lungs. Signs and symptoms of systemic poisoning include headache, nausea and vomiting, diarrhea, anorexia, anemia - exposure to high concentrations can result in cardiovascular and CNS effects.

Comparative Toxicity Data

(Inhalation Exposure)

Species	LC_{50} (mg/m^3)	Exposure Duration (Min)
Mouse	120	(10)
Rat	80	(10)
Guinea Pig	170	(10)
Rabbit	90	(10)
Dog	60	(10)
Goat	190	(10)
Monkey	80	(10)

(Dermal Exposure)

Species	LD_{50} (mg/kg)
Mouse	≈ 90
Rat	≈ 20
Rabbit	≈100

(Parenteral Exposure)
LD_{50} (mg/kg)

	Route		
Species	i.v.	i.p.	s.c.
Mouse	3-9		20-30
Rat	1-3		2-5
Rabbit	1-5		20-30

Delayed Toxicity: **(Acute Exposure)**

In addition to acute effects, there is also the possibility of "delayed effects" emerging some time after HD exposure. Delayed effects include: keratitis, respiratory alterations (e.g., bronchitis, emphysematous changes).

Pharmacokinetics/Toxicokinetics:

Sulfur mustard is readily absorbed from mucosal and skin surfaces. The bioconversion of HD has not been extensively studied - attempts to address this issue was reported in two studies conduced in the 1960s. Various metabolites are formed on bioconversion of HD which are mainly excreted in the urine. Sulfur mustard metabolites form water soluble conjugation products; primary metabolic derivatives of HD may undergo secondary biotransformation. HD has a very low detoxification rate and repeated small doses are cumulative.

Mechanism of Action:

HD acts first as a cell irritant and finally as a cellular poison and is particularly toxic to mitotic cells. HD is a classic alkylating agent and readily reacts with proteins, DNA and RNA, Cystostasis, mutation, and slow cell death can result.

Skin and Eye Irritation:

Sulfur mustard is a severe irritant/escharotic and a highly potent vesicant.

Long-Term Toxicity Effects:

Repeated exposure to HD can result in sensitization, chronic lung dysfunction (e.g., cough, shortness of breath, chest pain). In animal studies, subchronic effects [decreased body weight, epithelial hyperplasia (forestomach)] were manifest in rats following a 13-week exposure to sulfur mustard via gavage.

Reproductive Toxicity/Teratogenicity:

Several animal studies have investigated the potential of sulfur mustard to induce teratogenic and reproductive effects. Pregnant rats were exposed to HD and reproductive effects were noted. Major fetal malformations were not manifest; however, minor anomalies (i.e., misaligned sternebrae) were evident. Rabbit reproductive and teratogenicity studies on HD were negative. Results form a two-generation reproductive studies in rats treated with HD also indicate that sulfur mustard has little effect on reproductive performance and fertility. Reproductive

effects studies on occupational populations exposed in chemical warfare agent factories were inconclusive. Incidence from both human and animal studies regarding the reproductive toxicity of HD is generally negative.

Carcinogenicity/Tumorigenicity:

Chronic exposure to sulfur mustard can cause cancer of the respiratory tract, skin and blood forming tissues. Sulfur mustard has been found to be carcinogenic in laboratory studies. Retrospective epidemiological studies [mustard gas production workers (British, Japanese) and World War I war casualties] were conducted to ascertain the link between acute or chronic HD exposure to cancer risk. IARC overall evaluation: group I carcinogen. Evidence for carcinogenicity to humans (sufficient). Evidence for carcinogenicity for animals (limited).

Genotoxicity:

Sulfur mustard has been found to be highly mutagenic in a variety of microbial and mammalian mutagenicity assay systems.

Bioassay	**Results**
- Ames test (Salmonella typhimurium)	(Pos)
- Neurospora assay (Neurospora crassa)	(Pos)
- Saccharomyces assay (Saccharomyces cerevisive)	(Pos)
- HGPRT Assay [chinese hamster ovary (CHO)]	(Pos)
- Drosophila (dominant lethal)	(Pos)
- DNA Damage	
(mouse lymphoma)	(Pos)
(human cells, HeLa)	(Pos)
- Chromosomal Aberration (CA) [chinese hamster ovary (CHO) cells]	(Pos)
- Sister Chromatid Exchange (SCE) [chinese hamster ovary (CHO) cells]	(Pos)

Environmental Fate and Effects:

Sulfur mustard can undergo hydrolysis and volatilization - some leaching should also occur. In aqueous media, HD rapidly hydrolyzes but only when it is dissolved which is at very low

concentrations. Despite its high rate of hydrolysis, undissolved HD may persist for quite some time. In the atmosphere, HD vapor will degrade via reaction with photochemically-produced hydroxyl radicals. Hydrolysis products are chlorohydrin and thiodiglycol.

Hazard Categories and Lists:

IARC Group I Carcinogen.

Safety Numbers/Risk Estimate Numbers[a]:

Workplace: (8 hr) $mg/m^3 = 3X10^{-3}$
General population: $mg/m^3 = 3X10^{-4}$

References:

Anslow, W.P. and Houck, C.R., NDRC vol 1, Chapt 22, pp 440-478, 1946 (Unclassified).
Anslow, W.P. etal, J. Pharmacol Exp Therp, 93, 1-9 (1948).
Banks ,T.E., etal, Biochem J. , 40, 734-736 (1946).
Bouder, N.M., EATR-332, Oct 1940 (Unclassified).
Gates, M. and Moore, S., NDRC, Chapt 5, pp 30-58, 1946 (Unclassified).
Graef, I. etal, Am.J. Pathol., 24(1), 1-47 (1948).
Philips, F.S., Pharmacol Rev , 281-323 (1950).
Chemical Agent Data Sheets, EO-SR-7400, 1974.
MSDS (Sulfur Mustard)
MEDLINE/TOXLINE/TOXNET

[a] Values are Control Limits established by U.S. Surgeon General's Work Group-refer to Federal Register, 53(50), March 15, 1988.

TOXICITY PROFILE: NITROGEN MUSTARD (HN-1, HN-3)

The following is a synopsis of the toxicologic/pharmacologic characteristics of nitrogen mustard (HN-1, HN-3; HN-2 not discussed) - once considered gas warfare agent, other uses as antineoplastic agent and chemosterilant; toxicity similar to that of HN-1 and HN-3. The data base was retrieved via manual and on-line searches.

Chemical and Physical Properties

CAS Registry No.:

(HN-1)	**(HN-3)**
538-07-8	**555-77-1**

Chemical Name Synonyms:

bis-(2-chloroethyl) ethylamine	tris(2 -chloroethyl)amine
ethylbis(2-chloroethyl)amine	tri(2-chloroethyl)amine
	2,2',2"-trichlorotriethylamine

Trade Name and Synonyms:

HN-1	HN-3
NH-Lost	TL145
	Nitrogen Mustard-3

Mol Formula:

$C_6H_{13}Cl_2N$	$C_6H_{12}Cl_3N$

Structural Formula:

$$CH_3CH_2\text{-}N\begin{cases} CH_2CH_2Cl \\ CH_2CH_2Cl \end{cases} \qquad ClCH_2CH_2\text{-}N\begin{cases} CH_2CH_2\text{-}Cl \\ CH_2CH_2\text{-}Cl \end{cases}$$

Mol Wt: (170) (204)

Nitrogen mustard (HN-1) is an oily, colorless to pale yellow liquid with a musty odor. HN-1 is sparingly soluble in water and is freely soluble in acetone and other organic solvents. HN-1

hydrolyzes slowly because of low solubility in water - less readily hydrolyzed than HD. Hydrolysis products of HN-1 include hydroxyl derivatives and assorted condensation products.

Nitrogen mustard (HN-3), in its pure form, is an odorless liquid of low volatility. HN-3 is soluble in ether, benzene, and acetone and insoluble in water. The rate of hydrolysis is very slow because of low solubility in water. Hydrolysis products of HN-3 are hydrochloric acid and triethanolamine in dilute solutions. Dimer formation is possible at higher concentrations. HN-3 decomposes on heating and under certain conditions may polymerize.

Health Hazard Data/Health Effects Data

Target Organs:

Eyes, skin, gastrointestinal tract, blood forming organs, nervous system.

Acute Toxicity:

The nitrogen mustards are similar to sulfur mustard in their properties and biological effects. HN-1, however, is more volatile and less persistent than HD having about 1/5 the vesicant potency. HN-3, more stable than HN-1, is the main representative of the nitrogen mustards with vesicant properties similar to HD. The nitrogen mustards produce cytotoxic actions in a variety of tissues. Most symptoms are delayed for 4 to 6 hrs - eye irritation develops immediately. Nitrogen mustards act more quickly on the eyes. Effects on the respiratory system are essentially similar to that produced by HD. Systemic toxicity involves the gastrointestinal tract, blood forming tissue, hymphoid tissues, and nervous system. HN-3 is a potent convulsant. It has been reported that workers exposed briefly to nitrogen mustard in concentrations estimated between 10-100 ppm manifested nausea, vomiting and dilated pupils. In human volunteers doses of HN by the oral route resulted in nausea, vomiting, and diarrhea - larger doses can produce nervous system effects. The body does not readily detoxify nitrogen mustards; therefore, their actions are cumulative.

Toxicity Data

(Inhalation) mg/m^3

Species	HN-1	HN-3	Exposure Duration (Min)
Mouse	~90	~ 50	(10)
Rat	~70	≃ 60	(10)
Guinea Pig	~200	~ 200	(10)
Rabbit	~100	≃ 50	(10)

(Dermal) mg/kg

Species	HN-1	HN-3
Mouse	13	7-20
Rat	11-17	2-10
Rabbit	≃ 15	7-19
Guinea Pig	(-)	20

Parenteral LD_{50} (mg/kg)

Species	i.v.		s.c.	
	HN-1	HN-3	HN-1	HN-3
Mouse	(-)	1-2	1-2	2-6
Rat	0.5	0.7	1	2-5
Guinea Pig	(-)	(-)	(-)	(-)
Rabbit	~2	2	(-)	2

Skin and Eye Irritation:

Nitrogen mustards are severe eye irritants - severe exposure may cause exfoliation of the corneal epithelium. HN-1 and HN-3 are potent vesicants - the skin lesions are similar to those caused by sulfur mustard.

Long-Term Toxicity:

Chronic physiologic effects include scarring of the cornea, discoloration and atrophy of the iris. Repeated skin lesions leads to hypersensitivity of the skin.

Mechanism of Action: As potent alkylating agents, chloroethylamines and their metabolites react readily with amino, carboxyl, sulfhydryl, and phosphate groups of proteins and nucleic acids.

Pharmacokinetic/Toxicokinetics:

Nitrogen mustards are not readily detoxified.

Reproductive Toxicity/Teratogenicity:

Nitrogen mustard reported as embryo toxic in rats and also causes fatal abnormalities in rats.

Carcinogenicity/Tumorigenicity:

Nitrogen mustards are considered suspect carcinogens.

Genotoxicity:

The nitrogen mustards have been found to be genotoxic in a variety of microbial and mammalian genotoxic assays.

Environmental Fate and Effects:

Nitrogen mustard can undergo hydrolysis, less readily hydrolyzed than sulfur mustard.

Hazard Categories and Lists:

IARC Group 2A Carcinogen

Safety Numbers:

Control Limits have not been established for nitrogen mustards.

Tolerable environmental concentrations to general population (no data).

References:

Anslow, W.P. and Houck, C.R., NDRC vol 1, Chapt 22, pp 440-478, 1946 (Unclassified)
Anslow, W.P. and Karnofsky, D.A., J. Pharmacol. Exp. Therp., 91, 224-235 (1947).
Cope, A.C. , Gates, M. , and Renshaw, B. NDRC, Vol 1, Chapt 6, pp 59-82 , 1946 (Unclassified).
Graef, I. etal, Am. J. Pathol., 24 (1), 1-47 (1948).
Philips, F.S., Pharmacol Rev, pp 281-323, (1950).
Chemical Agent Data Sheets, EO-SR-7400 (1974).
MEDLINE/TOXLINE/TOXNET

TOXICITY PROFILE: LEWISITE (L)

The following is a synopsis of the toxicologic/pharmacologic characteristics of lewisite. The data base was obtained via manual and on-line searches.

Chemical and Physical Properties

CAS Registry No.: 541-25-3

Chemical Name Synonyms: arsine,dichloro(2-chlorovinyl)-
dichloro-(2-chlorovinyl)arsine
2-chlorovinyldichloroarsine
chlorovinylarsine dichloride

Trade Name and Synonyms:

L-1
M-1

Mol Formula: $C_2H_2AsCl_3$

Structural Formula:

```
              Cl
             /
ClCH=CH-As
             \
              Cl
```

Mol Wt: 207

Lewisite, an organic arsenical war gas, is a colorless to brownish, oily liquid with a geranium-like odor. Closely related analogs to lewisite are di-(2-chlorovinyl)chloroarsine(L-2) (CAS #40334-69-8) and tris-(2-chlorovinyl)arsine(L-3) (CAS #40334-70-1). Lewisite is a very reactive compound due to the presence of chlorine, carbon, multiple bonds, and trivalent arsenic. Lewisite is soluble in organic solvents and oils; insoluble in water and dilute acids. Lewisite has a complex high hydrolysis rate; however, the low solubility of L limits its hydrolysis. A major product of hydrolysis is the stable water soluble derivative 2-chlorovinylarsonous acid. Lewisite is oxidized to 2-chlorovinylarsonic acid in aqueous solution by many oxidants.

Health Hazard Data/Health Effects Data

Target Organs:

Skin, eyes, lungs, gastrointestinal tract, blood forming organs, central nervous system.

Acute Toxicity:

Lewisite is a vesicant which is highly toxic. The clinical manifestations of lewisite intoxication are similar to those caused by sulfur mustard. As a systemic poison, lewisite causes pulmonary edema, gastrointestinal effects, altered hemodynamics, changes in capillary permeability and associated functional disturbances, and nervous system effects. In severe systemic poisoning, shock and death result. Moist tissues (e.g., eyes, respiratory tract) are particularly affected by lewisite - unlike sulfur mustard lewisite causes an immediate searing sensation in the eye. Lewisite produces an immediate and strong stinging sensation to the skin - it is a severe irritant/escharotic and vesicant material. Lewisite is highly irritating to the respiratory tract - the respiratory lesions are similar to those produced by mustard.

Comparative Toxicity Data

(Inhalation Exposure)

Species	LC_{50} (mg/m^3)	Exposure Duration (Min)
Rat	≈ 150	(9)
Mouse	≈ 150	(10)
Guinea Pig	≈ 100	(9)
Rabbit	≈ 120	(10)

(Dermal Exposure)

Species	LD_{50} (mg/kg)
Mouse	15
Rat	15-24
Rabbit	5-6
Guinea Pig	12

(Parenteral Exposure)

LD_{50} (mg/kg)

Species	Route i.v.	Route i.p.	Route s.c.
Mouse	(-)	(-)	(-)
Rat	(-)	(-)	1
Rabbit	0.5-2.0	(-)	2
Guinea Pig	(-)	>2 mg/kg	1 mg/kg

Pharmacokinetic/Toxicokinetics:

Lewisite is absorbed through the skin and absorption by the lungs or G.I. tract as well. The body does not detoxify lewisite; however, British Anti-Lewisite (BAL,2,3-dimercapto-propanol is a very effective antidote against lewisite.

Mechanism of Action:

Lewisite toxicity demonstrated to involve interactions with cellular thiols and subsequent inhibition of energy pathways (cellular bioenergetics).

Skin and Eye Irritation:

Lewisite produces severe chemical burns on contact with tissue. Liquid arsenical vesicants produce severe damage to the eye. On contact, pain and blepharospasm occur immediately. Edema of the conjunctivae and lids occur rapidly. The corneal injury, which varies with the severity of exposure, may heal without scarring. Lewisite is a potent vesicant - stinging pain is noted within seconds following contact.

Long-Term Toxicity:

Reproductive Toxicity/Teratogenicity:

Lewisite administered to pregnant rats did not cause reproductive nor teratogenic effects. In rabbits, lewisite was associated with maternal toxicity and reproductive toxicity; however, teratogenicity was not observed in the fetuses.

Carcinogenicity/Tumorigenicity:

Although the evidence of the carcinogenicity of lewisite is equivocal; lewisite is generally considered a suspected carcinogen.

Genotoxicity:

Mutagenic potential of lewisite has been evaluated in microbial and non-microbial genotoxic assays. No mutagenic response was shown using the Ames assay. Lewisite was negative for genotoxic effects in the Drosophila assay.

The mutagenicity data suggest that lewisite is not mutagenic.

Environmental Fate and Effects:

Very limited environmental fate/effects data on lewisite. Lewisite is soluble in water and undergoes a rapid and reversible reaction - products of hydrolysis are lewisite oxide and hydrochloric acid.

Hazard Categories and Lists:

Not listed

Safety Numbers/Risk Estimates:

Control Limits[a]:

Workplace (8 hr) mg/m^3: $3X10^{-3}$
General population: mg/m^3: $3X10^{-3}$

References:

Bouder, N.M., EATR-332, Oct 1940 (Unclassified).
Gates, M., Williams, J.W., and Zapp, J.A. , NDRC Vol 1, Chapt 7, pp 83-114, 1946 (Unclassified).
Goldman, M. and Dacre, J.C., Rev. Environ. Contam. and Toxicol., 110, pp 75-115, 1989.
Chemical Agent Data Sheets, EO-SR-7400, 1974.
MSDS (lewisite)
MEDLINE/TOXLINE/TOXNET

[a] Values established by the U.S. Surgeon General's Work group-refer to Federal Register, 53(50), March 15, 1988.

APPENDIX B

TOXICOLOGIC PROPERTIES OF AGENT (HD, HN, L) DEGRADATION PRODUCTS

TOXICITY PROFILE: AGENT DEGRADATION PRODUCTS

The following is a brief summary of the toxicology data base on various agent (HD, HN, L) degradation products.

- **HD Sulfoxide**:

Physico-Chemical Properties:

Chemical Name and Synonyms: Sulfoxide, bis(2-chloroethyl)

Mol. wt: 175

Mol. Formula: $C_4H_8Cl_2OS$

Structural Formula: $ClCH_2CH_2S(=O)CH_2CH_2Cl$

Prepared by oxidation of sulfur mustard; somewhat soluble in water; readily soluble in organic solvents; little if any hydrolysis; hydrolyzed by alkali.

Health Hazards Data:

Although less toxic than sulfur mustard, HD sulfoxide retains the systemic toxicity characteristics of mustard. HD sulfoxide manifests skin irritant effects; however, the compound is non-vesicant.

- mouse s.c. LD_{50} (>125 mg/kg)
- mouse i.p. LD_{50} (100 mg/kg)

- **Divinyl Sulfoxide**:

Divinyl sulfoxide is the di-unsaturated analog of HD sulfoxide. Toxicity characteristics similar to that of HD sulfoxide.

- **HD Sulfone**:

CAS Registry No: **471-03-4**

Physico-Chemical Properties:

Chemical Name and Synonyms: Sulfone, bis(2-chloroethyl), bis(2-chloroethyl)sulfone, HD sulfone, mustard sulfone, TL4

Mol. wt: 191

Mol. Formula: $C_4H_8Cl_2O_2S$

Structural Formula:

```
     O   O
      \\ //
ClCH2CH2SCH2CH2Cl
```

Prepared from sulfur mustard via oxidation; colorless, odorless crystals; sparingly soluble in water; soluble in alcohol, and also in ether, chloroform.

Health Hazards Data:

HD sulfone is a highly poisonous derivative of sulfur mustard with vesicant action. Toxicity similar to that of HD - target organs respiratory tract, GI tract, CNS and blood forming tissues. Severe eye and skin irritant, vesicant.

- mouse s.c. LD_{50} (35 mg/kg)
- mouse inhalation LD_{50} (> 1000 mg/m^3, 10 min)
- rat s.c. LD_{50} (50 mg/kg)
- rabbit inhalation LCLo (1430 mg/m^3, 10 min)
- guinea pig s.c. LD_{50} (50 mg/kg)

- **Divinyl Sulfone**:

CAS Registry No: **77-77-0**

Physico-chemical Properties:

Chemical Names/Synonyms:

Mol. wt: 118

Mol. Formula: $C_4H_6O_2S$

Structural Formula:

```
      O   O
       \\ //
CH2=CHSCH=CH2
```

Clear, colorless liquid prepared by oxidation of sulfur mustard.

Health Hazards Data:

Divinyl sulfone is a vesicant with appreciable systemic toxicity. A derivative of sulfur mustard, divinyl sulfone elicits effects similar to that of the parent compound sulfur mustard. Target organs include GI tract, respiratory tract, and nervous system.

- mouse s.c. LD_{50} (16 mg/kg)
- rabbit i.m. LDLo (10-20 mg/kg)
- rabbit dermal LD_{50} ($\approx$ 20 mg/kg)

- **Trichloro HD (and Other Chlorinated Derivatives)**:

Derivative	Chemical Name	M.W.
trichloro HD	sulfone-β-chloroethyl α,β dichloroethyl	193
tetrachloro HD	sulfide,β-chloroethyl α,α,β trichloroethyl	227
	sulfide,β-chloroethyl α,β,β,trichloroethyl	227
hexachloro HD	α,α,β,β,β,β hexachloroethyl sulfide	296

Physico-Chemical Properties:

The higher chlorinated derivatives of sulfur mustard are oily liquids that are less soluble than HD.

Health Hazards Data:

The higher chlorinated derivatives of sulfur mustard are generally less toxic [i.e., hexachloro HD: mouse, s.c. LD_{50} (> 350 mg/kg) than sulfur mustard (mouse, s.c. LD50 $\approx$ 20 mg/kg) and possess no vesicant activity. These derivatives; however, do retain skin irritant action.

- **Sulfide, β-chloroethyl,β-chlorovinyl (and other vinyl analogs)**

Derivative	Structural Formula	M.W.
sulfide,β-chloroethyl,β-chlorovinyl	$ClCH_2CH_2SCH{=}CHCl$	157
sulfide,β-chloroethyl,α-chlorovinyl	$ClCH_2CH_2SCCl{=}CH_2$	157
sulfide,β-chloroethyl, vinyl	$ClCH_2CH_2SCH{=}CH_2$	122
sulfide,β-chloroethyl,α,β-dichlorovinyl	$ClCH_2CH_2SCCl{=}CHCl$	191

Physico-Chemical Properties:

Essentially similar to sulfur mustard but may vary due to insertion of the double bond and the degree of chlorination.

Health Hazard:

The above vinyl containing derivatives of sulfur mustard retain the systemic toxicity characteristics of HD. Generally, the presence of the double bond (vinyl group) may enhance the toxicity of the material. Of the above vinyl containing derivatives, only the β-chloro ethyl, β-chloro-vinyl sulfide derivatives are vesicant. All these vinyl containing analogs possess skin irritant properties.

Physico-Chemical Properties:

- **HN-1 Oxide**:

  - **M.W.: 186**
  - **Mol. Formula:** $C_6H_{14}Cl_2NO$
  - Tertiary amine oxides can be isolated and more stable than secondary amine oxides which tautomerize.

Health Hazards Data:

  - Systemic toxicity similar to that of nitrogen mustard (HN-1).
  - mouse i.p. LD_{50} (50-100 mg/kg)

- **HN-3 Oxide:**

  - **M.W.:** 220
  - **Mol. Formula:** $C_6H_{13}C_{13}NO$
  - Tertiary amine oxides can be isolated and are more stable than secondary amine oxides which tautomerize.

Health Hazards Data:

  - Systemic toxicity similar to that of nitrogen mustard (HN-3).
  - mouse i.p. LD_{50} (2-5 mg/kg)

- **Lewisite Oxide**:

  Physico-Chemical Properties:

  Chemical Name/Synonyms: 2-chlorovinyl arsenious oxide

  Mol. wt: 152
  Mol. Formula: C_2H_20ClAs
  Structural Formula: CHCl=CHAs0

Lewisite oxide is a white crystalline powder which is sparingly soluble in water and alcohol. It is prepared by hydrolysis of lewisite.

Health Hazard Data:

Lewisite oxide is a potent respiratory irritant and vesicant.

- mouse s.c. lethality [2 mg/kg (0/5), 5 mg/kg (5/5), 10 mg/kg (5/5)]
- mouse s.c. LD_{50} ~ 3 mg/kg
- dog i.p. LDLo 2 mg/kg
- unspecified species LCLo 120 mg/m^3 (30 min)

° **2- chlorovinyl arsonic acid:**

Physico-chemical Properties:

Mol Wt: 186

Mol Formula: $C_2H_4O_3ClAs$

2-chlorovinyl arsonic acid is a solid prepared by hydrolysis of lewisite.

Health Hazards Data:

2-chlorovinyl arsonic acid is an irritant; however, it is not vesicant.

- mouse i.p. LD_{50} >500 mg/kg

References:

Bouder, N.M. EATR-332, Oct 1940 (Unclassified)
Stahmann, M.A. and Bergmann, M. J. Org. Chem., 11, 586-591, 1946.
Thomson, J.F. , etal, OSRD Rpt No. 5194, June 1945 (Unclassified).
Young, H.D., Geiling, E.M., and Cannan, R.K. , OSRD Rpt No. 4176, Oct 1944 (Unclassified).

APPENDIX C

TOXICITY PROFILES OF OXIDANTS UTILIZED IN THE CHEMICAL NEUTRALIZATION OF CAIS SETS

Toxicity Profile: 1,3-dichloro-5,5-dimethyl Hydantoin (DCDMH)

Toxicity Profile: 1,3-dibromo-5,5-dimethyl Hydantoin (Brom-55p)

Toxicity Profile: m-chloroperoxybenzoic acid (m-cpba)

Toxicity Profile: 1,3-Dichloro-5,5-dimethyl Hydantoin

The following is a brief summary of the toxicology database relevant to 1,3-dichloro-5,5-dimethylhydantoin. The toxicologic database was assessed via manual (scientific literature, handbook sources, MSDS) and on-line (RTECS, HSDB, TOXLINE/TOXNET) literature searches.

Chemical and Physical Properties

CAS Registry No.: 118-52-5

Synonyms: 1,3-dichloro-5,5-dimethyl-2,4-imidazolidin
Dichlorantin
DCDMH

DCDMH is a white powder with a mild chlorine odor. Presumably DCDMH is less corrosive than hypochlorite solutions with the same concentration of available chlorine. The pH of an aqueous solution is about 4.5. Solubility in water is 0.21%; in chloroform 14%. At pH 9 DCDMH decomposes completely.

Production and Use:

DCDMH is a chlorinating agent, disinfectant, industrial deodorant and is the active ingredient of powder laundry bleaches. DCDMH contains about 66% "available chlorine". Other uses of DCDMH are: (1) as an intermediate drug and insecticide manufacturing and (2) as a stabilizer and polymerization catalyst and (3) used as a chemical warfare decontaminating agent.

Routes of Entry: Dermal, inhalation

Health Hazard Data/Health Effects Data

Target Organs: Skin, lungs, gastrointestinal tract, central nervous system.

Acute Toxicity:

DCDMH is an irritant to eyes, skin, respiratory tract, and mucous membranes. DCDMH may cause dyspnea (labored breathing)-pulmonary edema may result following severe exposure.

Acute toxicities as follows:

Oral:

- rat oral LD50 (542 mg/kg; 1200 mg/kg)
- rabbit oral LD50 (1520 mg/kg)
- guinea pig oral LD50 (1350 mg/kg)

Dermal:

- rabbit dermal LDLo 20 g/kg (mortality 1/4)

Inhalation:

- rat 5/10 deaths [1-hr exposure, 20.5 mg/L (nominal) conc]

Skin and Eye Irritation: Severe skin and eye irritant.

Long-Term Toxicity Effects: Multiple dose toxicity data (oral): TDLo 8784 mg/kg/28-day.

Reproductive Toxicity/Teratogenecity:

The reproductive toxicity potential of DCDMH was evaluated in mice using the Chernoff/Kavlock preliminary development toxicity test. DCDMH exposure resulted in maternal toxicity and mortality but minimal toxic effects were noted in the offspring of treated animals.

Pharmacokinetics/Toxicokinetics: No information.

Genotoxicity:

DCDMH was evaluated in a number of genotoxicity assays: sex-linked recessive lethal (SLRL) mutations in Drosophila melanogaster; chromosomal aberrations (CA) and sister chromatid exchange (SCE) in Chinese hamster ovary cells (CHO assay); and Ames assay (Salmonella typhimurium).

The results are summarized as follows:

Assay	Results
Salmonella assay:	neg
Drosophila assay (SLRL):	pos
Chromosomal Aberrations (CA):	neg
Sister Chromatid Exchange (SCE):	neg

Carcinogenicity/Tumorigenicity: No information.

Environmental Fate and Effects: no information

Hazard Categories and Lists:

EPA TSCA Inventory: Yes
Extremely Hazardous Substance: No

Safety Numbers/Risk Estimates No.:

Threshold Limit Value (TLV/TWA): 0.2 mg/m^3
Permissible Exposure Limit (PEL): 0.2 mg/m^3
Short-term Exposure Limit (STEL): 0.4 mg/m^3

Transportation Data: extreme irritant/corrosive

References:

1. Bromatol. Chem. Toksykol 19(1):52-4 (1986).
2. Doc Threshold Limit Values, 5:183 (1986).
3. Environ. Mutagen, 5 (Suppl.):3-142 (1983).
4. Environ. Mutagen, 7:677-702 (1985).
5. Environ Mol. Mutagen 10 (Suppl 10):1-175 (1987).
6. Environ Mol. Mutagen, 14(4):245-251 (1989).
7. Gig Sanit 47(6):76-78 (1982).
8. HSDB
9. RTECS
10. Teratog Carcinogen Mutagen, 7:29-48 (1987).
11. The Merck Index, 9th Ed., Merck & Co, Inc., Rahway, N.J. (1976).
12. TOXLINE/TOXNET.
13. U.S. EPA 8EHQ-0281-0382.
14. U.S. EPA/OTS (Doc #88-8100228).
15. U.S. EPA/OTS (Doc #88-8100173).

Toxicity Profile: 1,3-Dibromo-5,5-dimethyl Hydantoin

The following summarizes the toxicity and health effects of 1,3-dibromo-5,5-dimethylhydantoin. The toxicology database was assessed via manual (scientific literature, handbook sources, MSDS) and on-line (RTECS, HSDB, TOXLINE/TOXNET) literature searches.

Chemical and Physical Properties

CAS Registry No.: 77-48-5

Synonyms: 5,5-dimethyl-1,3-dibromohydantoin
N,N'-dibromo-dimethylhydantoin
2,4-Imidazolidinedione, 1,3-dibromo-5,5-dimethyl
Dibromantin
DBH
Brom-55p

Production and Use: Industrial biocide, bactericide, disinfectant.

Routes of Entry: Dermal, inhalation

Health Hazards Data/Health Effects Data

Target Organs: Skin, lungs

Acute Toxicity:

Brom-55p is an irritant to eyes, skin, respiratory tract, and mucous membranes. Brom-55p may cause dyspnea (labored breathing).

Acute toxicities as follows:

Oral: rat oral LD50 (760 mg/kg).
Dermal: rabbit dermal LDLo 20 g/kg (mortality 1/6).
Inhalation: rat inhalation: 9/10 mortality (1-hr exposure, nominal conc 29.4 mg/L).

Skin and Eye Irritation: Severe skin and eye irritant.

Long-Term Toxicity Effects: Effects on thyroid gland in rats chronically exposed to dibromatin.

Reproductive Toxicity/Teratogenecity: No information.

Pharmacokinetics/Toxicokinetics: No information.

Genotoxicity: No information.

Carcinogenicity/Tumorigenicity: No information.

Environmental Fate and Effects: No information.

Hazard Categories and Lists:

EPA TSCA Inventory: Yes
Extremely Hazardous Substance: No

Safety Numbers/Risk Estimates:

Threshold Limit Value (TLV): No
Permissible Exposure Limit (PEL): No

Transportation Data:

Domestic DOT: extreme irritant/corrosive

References:

Gig Sanit, 36(10), 108-109 (1971)
HSDB
RTECS
TOXLINE/TOXNET
USEPA 8EHQ-0281-0382
USEPA/OTS Doc #88-8100228

TOXICITY PROFILE: m-CHLOROPERBENZOIC ACID

The following is a profile of toxicity characteristics of m-chloroperbenzoic acid (m-cpba). The toxicological data base was accessed via manual (scientific literature, handbook sources, MSDS) and on-line (RTECS, HSDB, TOXLINE/TOXNET) literature searches.

Chemical and Physical Properties:

CAS Registry No.: 937-14-4

Synonyms: 3-chloroperbenzoic acid
m-chloroperoxybenzoic acid
3-chloroperoxybenzoic acid
m-chlorobenzoyl hydroperoxide
m-CPBA

Chloroperbenzoic acid (m-CPBA), a peroxy compound, is a strong oxidizer (empirical formula ($C_7H_5C1O_3$); mol. wt (172); melting point 92°C at 760 mm Hg). Chloroperbenzoic acid is a white powder having a pungent odor and is slightly (0.1-1%) soluble in water. Decomposition products include chlorine, carbon monoxide, and carbon dioxide. Incompatibles: strong reducing agents, combustible materials.

Production and Use:

Chloroperbenzoic acid is primarily used as a laboratory reagent - its use in industrial processes is limited.

Routes of Entry: Inhalation, skin

Health Hazard Data/Health Effects Data:

Target Organs: Lung, eye, skin

Acute Toxicity:

Skin, mouse TDLo[1] = 21 g/kg

Effects on over exposure: Irritation of the upper respiratory tract, eye irritation, skin irritation

Long-term Toxicologic Effects: No data

Reproductive Toxicity: No data

Pharmacokinetics/Toxicokinetics: No data

Mutagenicity:

A number of mutagenicity studies (Ames Assay, UDS Assay) have been conducted on m-chloroperbenzoic acid/m-chloroperoxybenzoate[1,3]. m-chloroperbenzoic acid was negative in the Ames assay, m-choroperoxybenzoate gave a negative response in both the Ames and hepatocyte UDS assays.

Carcinogenicity/Tumorigenicity:

Peroxy compounds are sources of free radicals and are important in many industrial processes (e.g., synthesis, polymerization, curing). Peroxy compounds have also become the subject of toxicological interest in particular their role in cancer/tumor/mutation induction.

The tumor - promoting activity of m-CPBA has been established (m-cpba is a tumor promoter)[2,5]. The carcinogenic/tumorigenic activity of m-CPBA has not been established.

Environmental Fate and Effects: No data

Hazard Categories and Lists:

Extremely hazardous substance: No
CERCLA hazardous substance: No
SARA toxic chemicals: No
EPA TSCA inventory: Yes

Safety Numbers/Risk Estimates:

Threshold Limit Value (TLV/TWA): Not Established
Short-Term Exposure Limit (STEL): Not Established
Permissible Exposure Limit (PEL): Not Established

EPA Hazardous Waste No.: D001 Ignitable waste

Transportation Data:

Domestic DOT: Organic peroxide, solid, N.O.S.
Air (ICAO/IATA): Packing Group II.

[1] **TDLo = Toxic Dose Low "The lowest dose of a substance introduced by any route, other than inhalation, over any given period of time and expected to produce any toxic effect in human or to produce carcinogenic, neoplastigenic, or teratogenic effects in animals or humans."**

References:

1. Agric. Biol. Chem, 44:1675-1678 (1980).
2. Cacinogenesis, 12(4):563-569 (1991).
3. Environ. Mutagenesis, 3:11-32 (1981).
4. Hazardous Substances Databook (HSDB).
5. J. Natl. Cancer Inst., 55(6):1359-1361 (1975).
6. Material Safety Data Sheets (MSDS), J.T. Baker Inc. (1993).
7. Registry of Toxic Effects of Chemical Substances (RTECS).
8. Sax, Dangerous Properties of Industrial Materials, 6th ed, p 748, Von Nostrand Rinhald Co., N.Y. (1984).
9. TOXLINE/TOXNET.

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APPENDIX D

TOXICITY PROFILES OF SOLVENTS USED IN THE CHEMICAL NEUTRALIZATION OF CAIS SETS

Toxicity Profile: chloroform ($CHCl_3$)

Toxicity Profile: t-butanol (t-BuOH)

Toxicity Profile sulfolane

TOXICITY PROFILE: CHLOROFORM

The following is a brief summary of the toxicology data base pertinent to chloroform. Database obtained via manual and on-line literature searches.

Chloroform:

CAS Registry No: 67-66-3

Physico-Chemical Properties:

Chemical Name and Synonyms: methane, trichloro-
methyl trichloride
formyl trichloride
R20
Freon-20

Mol. wt: 119

Mol. Formula: $CHCl_3$

Chloroform is a clear, colorless volatile liquid with a pleasant, etheric odor. Vapor pressure is 100 mm Hg at 10°C. Water solubility is low and is soluble in carbon disulfide. Forms azeotropes with acetone, ethanol, n-hexane, methanol, and water. Oxidized by strong oxidizing agents (i.e, chromic acid) with formation of phosgene and chlorine.

Production and Use:

General solvent for adhesives and pesticides; solvent for fats, oils, rubbers, alkaloids, waxes; insecticidal fumigant; dry cleaning agent; chemical intermediate for dyes, pesticides; component of several over-the-counter medications/ointments.

Health Hazard Data/Health Effects Data:

Routes of Exposure:

Inhalation, dermal, oral

Target Organs:

Central nervous system, GI tract/liver, cardiovascular, kidneys.

Acute Toxicity:

Toxic effects may be encountered following exposure to chloroform. Aside from the irritant effects of $CHCl_3$, the range of acute effects on exposure to chloroform include headache, dizziness, nausea, CNS depression, cardiac arrhythmia, and death. Chronic exposure results in liver and kidney damage.

Clinical Effects:

Exposure to 1000 ppm of $CHCl_3$ for about 10 min can cause dizziness and GI upset, exposure to 14,000 ppm can result in CNS depression.

- **Ocular/dermatologic**: irritant
- **Respiratory**: pulmonary edema, chemical pneumonitis, respiratory depression
- **Neurologic**: CNS depression, headache
- **Gastrointestinal**: irritation, nausea, vomiting
- **Hepatic:** fatty infiltration, necrosis
- **Genitourinary**: renal damage

General Toxicity (LD_{50}/LC_{50}) Values:

rat, oral LD_{50}: 908 mg/kg
rat, inhalation LC_{50}: ≈ 50 g/m^3 (4 hr)
mouse, i.p. LD_{50}: 630 mg/kg
mouse, s.c. LD50: 704 mg/kg
mouse, inhalation LC50: 28 g/m^3
rabbit, oral LD50: 500 mg/kg
rabbit, inhalation: 59 g/m^3
rabbit, s.c. LD50: 800 mg/kg
rabbit, skin LD50: > 20 g/kg

Skin and Eye Irritation:

Chloroform is a skin and eye irritant.

Long-term Toxicity:

In mice, repeated exposure to $CHCl_3$ resulted in cirrhosis of the liver and hepatomas.

Reproductive Toxicity/Teratology:

Chloroform is not highly teratogenic; however, developmental abnormalities of the musculoskeletal system has been reported. Chloroform is highly embryotoxic.

Toxicokinetics/Pharmacokinetics:

Chloroform is well-absorbed via the respiratory system, absorbed via the GI tract, and to some extent via the skin. Following rapid uptake (pulmonary), $CHCl_3$ is distributed to all organs with relatively high concentrations in the nervous system. Redistribution of chloroform in body tissues can occur as a result of compound build-up in fatty tissues. The liver is the primary site for chloroform metabolism - other tissues (i.e., kidneys) can also metabolize $CHCl_3$. Chloroform is predominantly metabolized to CO_2 and to a very limited extend CO. Administered orally, for example, most or all of the dose is eliminated unchanged via the lungs in expired air. There is a possibility for the urinary excretion of chloroform metabolites (i.e., urea).

Mutagenicity:

Ames assay: findings equivocal

Sister Chromatid Exchange SCE (human lymphocytes): Neg
Sister Chromatid Exchange SCE (hamster embryo cells): Neg

Carcinogenicity/Tumorigenicity:

Overall evaluation: suspect carcinogen

Hepatomas and hepatocellular carcinomas in mice on repeated exposure. NCI carcinogenicity bioassay (rat) indicated increased epithelial tumors of the kidney. Results of various rodent bioassays indicate that $CHCl_3$ is carcinogenic/tumorigenic. Carcinogenic/neoplastic by RTECS criteria. IARC classification group 2B (inadequate evidence of carcinogenicity in humans, sufficient evidence of carcinogenicity in animals).

Ecotoxicity and Environmental Fate/Effects:

Terrestrial Fate: When spilled on land, $CHCl_3$ is expected to evaporate rapidly into the atmosphere. It is poorly absorbed onto soil. It can leach into the ground water.

Aquatic Fate: When released in water, $CHCl_3$ is primarily lost via evaporation - it can be absorbed to sediment.

Biodegradation/Abiotic Degradation: Conflicting data on the biodegradation of chloroform. Slow but substantial biodegradation in the presence of proper microbial populations. Chloroform has a negligible rate of hydrolysis.

Aquatic Toxicity:

LC_{50} (rainbow trout):	43,800 $\mu g/L$ (96 hr)
LC_{50} (blue gill):	100,000 $\mu g/L$ (96 hr)
LC_{50} (Daphnia magna):	28,900 $\mu g/L$ (48 hr)

Hazard Categories and Lists:

CERCLA hazardous substance: Yes
EPA TSCA inventory: Yes
RCRA waste: Yes

Safety Numbers/Risk Estimates:

TLV/TWA ≈ 50 mg/m^3 (10 ppm)
OSHA standard: 8 hr time-weighed avg - 10 mg/m^3 (2 ppm)
STEL - deleted

Transportation Data:

Domestic transportation: primary hazard class ORMA [other regulated material - material that has an anesthetic, noxious, toxic or other similar property].

References:

Registry of Toxic Effects of Chemical Substances (RTECS)
Hazardous Substances Data Book (HSDB)
TOXLINE/TOXNET
Sax, Dangerous Properties of Industrial Materials, 7th Edition

TOXICITY PROFILE: t-butyl alcohol

The following is a brief summary of the toxicology data base pertinent to t-butyl alcohol. Database obtained via manual and on-line literature searches.

t-butyl alcohol:

CAS Registry No: 75-65-0

Physico-Chemical Properties:

Chemical Name and Synonyms:
2-methyl-2-propanol
1,1 dimethyl ethanol

Mol. wt: 74

Mol. Formula: $C_4H_{20}O$

t-butyl alcohol esists as a colorless liquid or as colorless hygroscopic crystals (m.p. 25°C, b.p. 82.9°C). Sp.G.: 0.78 at 20°C, v.p.: 31 torr at 20°C. Completely miscible with water, miscible with alcohol and ether.

Production and Use:

t-butyl alcohol is used in the manufacture of flotation agents, flavors, perfumes, used extensively as a solvent, as a gasoline additive, strong mineral acids cause decomposition.

Health Hazard Data/Health Effects Data:

Routes of Exposure:

Inhalation

Target Organs:

Central nervous system

Acute/General Toxicity:

Signs of intoxication are similar to those of the other butyl alcohols (i.e. ataxia and narcosis) results in liver and kidney damage.

Acute/General Toxicity (con't)

rat oral LD50: 3500 mg/kg
mouse i.v. LD50: 1538 mg/kg
rabbit oral LD50: 4500 mg/kg

Skin and Eye Irritation:

Skin and eye irritation and hyperemia

Long-term Toxicity:

Sub-chronic inhalation study details not reported.

Reproductive Toxicity/Teratology:

Teratology assessment of t-butyl alcohol in rats via inhalation exposure. The highest concentrations were maternally toxic. A dose-dependent reduction in fetal weight was noted, no teratologic effects were seen.

Toxicokinetics/Pharmacokinetics:

Genotoxicity:

Salmonella assay: negative
Mouse lymphoma assay: negative
Chinese hamster ovary (CHO) assay: negative

Carcinogenicity/Tumorigenicity:

NTP has conducted several rodent (rat, mouse) carcinogenisis assays (test material incorporated in drinking water). The rat study was considered inadequate, data not reported. Final report of the mouse study has not been completed.

Safety Numbers/Risk Estimates:

TLV/TWA: 303 mg/m^3 (100 ppm)
TLV-STEL: 455 mg/m^3 (150 ppm)

Transportation Data:

DOT Classification: Flammable liquid

References:

Registry of Toxic Effects of Chemical Substances (RTECS)
Hazardous Substances Data Book (HSDB)
TOXLINE/TOXNET
Sax, Dangerous Properties of Industrial Materials, 6th Edition
ACGIH, Documentation of TLV's, 6th Edition
Fund. And Appl. Toxicol., 12:469-479 (1989)

TOXICITY PROFILE: SULFOLANE

The following is a brief summary of the toxicologic and biologic effects of Sulfolane. Toxicological data was derived manually from the scientific literature, handbook sources, MSDS's and from on-line data bases such as RTECS, HSDB, TOXLINE/TOXNET, etc.

Chemical and Physical Properties

CAS Registry No.: 126-33-0

Synonyms: Tetrahydrothiophene-1,1-dioxide
Thiolane-1,1-dioxide
Thiophan sulfone

Sulfolane, a clear colorless liquid with a pungent odor, is a highly polar solvent. Boiling point: 285°C (545°F) at 760 mm Hg; melting point: 8°C (46°F) at 760 mm Hg. Sulfolane has low vapor pressure (0.0062 mm Hg at 27°C). Specific gravity of the material is 1.26. Sulfolane is miscible with water, acetone; partially miscible with various organics; freely soluble in alcohol. Sulfolane is soluble in dilute mineral acids. It is incompatible with strong oxidizing agents. Decomposition products include oxides of sulfur, CO_2, and CO.

Production and Use:

Sulfolane is produced from the catalytic hydrogenation of sulfolene oxides. Commercially available as anhydrous sulfolane and as sulfolane containing 3% water. Uses are as follows: solvent in extraction processes, polymerization solvent, as a plasticizer, hydraulic fluid component, used in textile finishing, curing agent (epoxy resins) and as an antibacterial agent.

Routes of Entry: dermal, inhalation, oral

Health Hazards Data/Health Effects Data:

Target Organs: Eye, skin, respiratory tract, liver, gastrointestinal tract, central nervous system.

Acute Toxicity:

Effects of over-exposure include nausea and vomiting, respiratory and gastrointestinal irritation, and central nervous system depression.

LD50 VALUES

Route	Rat	Mouse	Guinea Pig	Rabbit
		Species		
Oral	2,100 mg/kg •1,500 mg/kg	•2,000 mg/kg	1,815 mg/kg	(-)
S.C.	1,606 mg/kg	1,360 mg/kg	(-)	1,900 mg/kg
I.P.	1,598 mg/kg	1,250 mg/kg	1,331 mg/kg	(-)
I.V.	1,094 mg/kg	1,080 mg/kg	(-)	640 mg/kg
Dermal	>3,800 mg/kg	(-)	(-)	3,000- 4,000 mg /kg
Inhalation	4,700 mg/m^3[a]	(-)	(-)	(-)

[a] LCLo

Skin and Eye Irritation:

Mild skin irritant, free of sensitizing properties; mild eye irritant

Long-Term Toxicity Effects:

A number of repeat-dose inhalation studies have been conducted in various species (guinea pigs, dogs, and monkeys) to ascertain the effects of repeat-dose exposure to sulfolane [14]. Long-term toxic effects included primarily central nervous system effects (convulsions, aggressive behavior), gastrointestinal (vomiting); and respiratory (inflammation, hemorrhaging).

Reproductive Toxicity: No information available.

Pharmacokinetics/Toxicokinetics:

Sulfolane is excreted both unchanged and as 3-hydroxy-sulfolane.

Mutagenicity:

The genotoxicity of sulfolane has been assessed in a number of mutagenicity bioassays.

I. Microbial Assays: S. typhimurium (Ames assay): (negative)
- E. coli: Negative
- S. cerevisiae: Equivocal

II. Mammalian Assays:
- Chromosomal aberration (CA): Negative [rat hepatocytes)

Carcinogenicity/Tumorigenicity: No information available.

Environmental Fate and Effects:

Stable in soil and aquatic environments - biodegradation minimal. Sulfolane is highly mobile in soil.

Volatilization minimal because of high water solubility and low vapor pressure.

Hazard Categories and Lists:

Extremely hazardous substance: No
CERCLA hazardous substance: No
TSCA Inventory: Yes

Safety Numbers/Risk Estimates:

TLV/TWA: Proposed (5 ppm)
PEL: Not established
STEL: Not established

EPA Hazardous Waste No.:

Transportation Data: N.O.S. (Non-regulated)

References:

1. Am Ind Hyg Assoc. J., 30, 470-476 (1969).
2. Arch. Int. Pharmacodyn, 119 (3-4), 423-434 (1959).
3. Aquatic Pollutants. Transformation and Biological Effects. p283-98. Hutzinger, O. et al (eds). Pergamon Press. Oxford (1978).
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5. CRC Handbook of Data on Organic Compounds. Vols I and II. V2 343. CRC Press Inc., Boca Raton, FL (1985).
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7. EPA/OTS DOC #0484-0304
8. Handbook of Environmental Data of Organic Chemicals, 2nd Ed. p 1060. Van Nostrand Reinhold Co., New York, NY (1983).
9. Hawley's Condensed Chemical Dictionary. 11th Ed. P 1106. Van Nostrand Reinhold Co., New York, NY (1987).
10. Hazardous Substances Databank (HSDB).
11. Kirk-Othmer Encyclopedia of Chemical Technology, 3rd Ed. Vol 21, pp 961, 963. 1978-1984. John Wiley and Sons. New York, NY (1983).
12. Organic Solvents. 4th Ed. Vol2. P686-87. John Wiley and Sons. New York, NY (1986).
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14. Res Comm Chem Path Pharmacol, 15(3), 571-80 (1976).
15. The Merck Index, Encyclopedia of Chemicals, Drugs and Biologicals. p1414. Merck and Company, Inc., Rahway, NJ (1989).
16. Toxicol Appl Pharmacol, 40(3), 463-470 (1977).
17. Toxline/Toxnet.
18. Waters Rsch, 13, 617-30(1979).

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APPENDIX E

PHYSICOCHEMICAL PROPERTIES OF THE TREATMENT REAGENTS, SOLVENTS, AND AGENTS

Physicochemical Properties of Sulfur Mustard (HD), Nitrogen Mustard (HN) and Lewisite (L)

Physicochemical Properties of Oxidizers (Treatment Reagents)

Physicochemical Properties of Solvents

PHYSICOCHEMICAL PROPERTIES OF SULFUR MUSTARD (HD), NITROGEN MUSTARD (HN), AND LEWISITE (L)

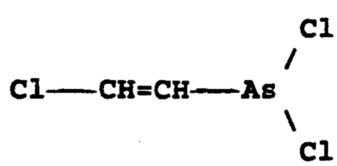
Sulfur Mustard, HD [bis(2-chloroethyl)sulfide]

CAS #505-60-2
MW: 159.
Oily, amber to colorless liquid (b.p. 442°F).
Odor: like garlic or horseradish.
Vapor pressure: low (0.09 mm at 86°F).
Specific gravity/density: 1.27 g/ml.
Freezing point: 58°F, 14.5°C
Solubilities: Slightly soluble in water, soluble in organic solvents such as chloroform, acetone.
Reacts with oxidizing agents (peroxide, hypochlorite salts) to form sulfoxides and/or sulfones; reacts with alkali, ammonia or amines to form substitution products.

$$Cl\text{-}CH_2\text{-}CH_2\text{-}S\text{-}CH_2\text{-}CH_2\text{-}Cl$$

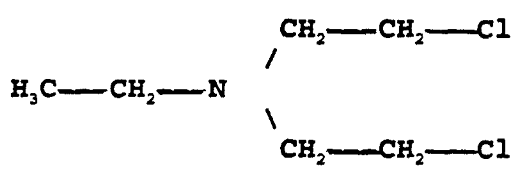
Lewisite, L [arsine, dichloro(2-chlorovinyl]

CAS #541-25-3
MW: 207
Colorless to brown liquid (b.p. 374°F).
Odor: like geraniums.
Vapor pressure: low (0.4 mm at 20°C)
Specific gravity/Density: 1.89 g/ml.
Freezing point: -0.2 to 9°F, -18 to -13°C.
Solubilities: insoluble in water, dilute mineral acids; soluble in organic solvents.
Slowly decomposed by water, hydrolyzed by alkalies, neutralized and inactivated by hypochlorite salts.



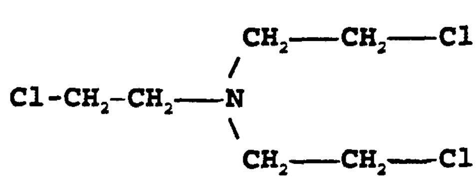
Nitrogen Mustard, HN-1 [bis(2-chloroethyl)ethylamine]

CAS #538-07-0
MW: 170.
Mol. Formula: $C_6H_{13}Cl_2N$
Oily, colorless to pale yellow liquid.
Odor: fishy, musty.
Vapor pressure: 0.24 mm at 77°F.
Specific gravity/density: 1.09 g/ml.
Freezing Point: -29°F, -34°C
Solubilities: Sparingly soluble in water, miscible with DMFA, freely soluble in alcohol, acetone, chloroform, and other organic solvents.
Forms a water soluble salt with acids; converted by oxidizing agents (peroxides or hypochlorites) to an amine oxide and other products.



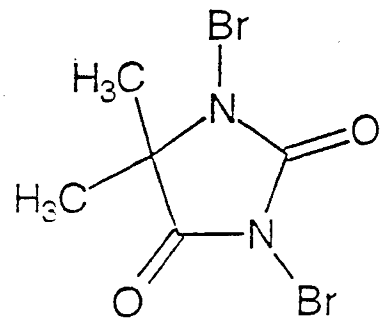
Nitrogen Mustard, HN-3 [tris(2-chloroethyl)amine]

CAS #555-77-1
MW: 204.
Mol Formula: $C_6H_{12}Cl_3N$
Oily liquid.
Odor: fishy, odorless when pure.
Vapor pressure: 0.011 mm at 77°F.
Specific gravity/density: 1.24 g/ml.
Freezing point: 25°F, -3.7°C.
Solubilities: Nearly insoluble in water, miscible with DMFA, soluble in chloroform, alcohol, acetone, and other organic solvents.
Forms water soluble salts with acids; reacts with oxidizing agents (peroxides or hypochlorites) to form an amine oxide and other products.



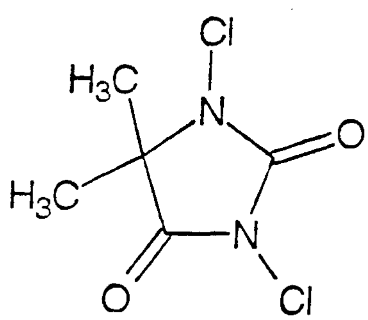
1,3-Dibromo-5,5-dimethylhydantoin (Brom 55-P, dibromantin)

CAS #77-48-5
MW: 285
Yellowish solid.
Vapor pressure: very low.
Melting point: 369-376°F, 187-191°C.
Solubilities: soluble in chlorinated and aromatic organic solvents, slightly soluble (water).
Odor: bromine-like.
Reacts with easily oxidizable organics to form oxidation or bromination products; slowly reacts with water to form bromine.



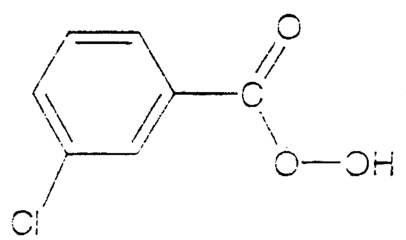
1,3-Dichloro-5,5-dimethylhydantoin (DCDMH dichlorantin)

CAS #118-52-5
MW: 197.
Specific gravity/gravity: 1.5 to 20°C.
Vapor pressure:
Melting point: 132°C.
Solubilities: pH aqueous salin (4.4), water (0.21%); chloroform (14%); benzene (9%).
Chloroform, benzene, toluene, reacts with alcohols.
Odor: mild chlorine odor.
Reacts with readily oxidizable organics to form oxidation or chlorination products; decomposes and conflagrates when heated to 414°F, decomposes at pH9.



m-Chloroperoxybenzoic acid (mcpba)

CAS #937-14-4
MW: 172
(M-chlorobenzoyl hydroperoxide)
White solid.
Composition: 50-70 percent mCPBA, contains water.
Vapor pressure: very low.
Melting point: 92°C at 760 mm Hg.
Solubilities: water: slight (0.1-1%).
Soluble in chloroform and other organic solvents.
Odor: pungent odor.
Reacts with sulfides and mercaptans, alcohols (except tertiary), aldehydes and ketones, amines, and other oxidizable compounds; reacts with inorganic iodides and sulfites.



$CHCl_3$

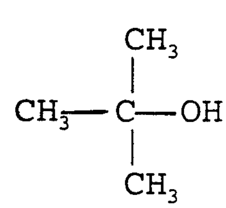
Chloroform

CAS #67-66-3
MW: 119
Colorless, mobile liquid (b.p. 143°F, 62°C).
Vapor pressure: 159 mm at 68°F.
Specific gravity/density: 1.48 g/ml.
Freezing point: -82°F, -63°C.
Solubilities: slightly soluble in water, miscible with most organic solvents.
Odor: sweetish.
Reacts slowly with air, in light, to form phosgene and hydrogen chloride (therefore, 0.7% ethyl alcohol is added as stabilizer); reacts with strong alkali (sodium hydroxide); reacts with amines.

t-BuOH

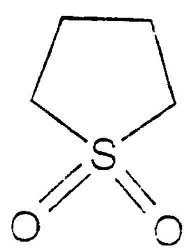
t-Butyl alcohol (2-methyl-2-propanol)

CAS #75-65-0
MW: 74
Colorless liquid (b.p. 82°C).
Vapor pressure: 44 mm at 79°F.
Specific gravity/density: 0.78 at 20°C.
Melting point: 78.3°F, 25.7°C.
Solubilities: soluble in water, miscible with alcohol, ether.
Odor: camphor-like.
Reacts with concentrated sulfuric acid or hydrochloric acids.



Sulfolane-W(with 3% H_2O) (tetrahydrothiophene)

CAS #126-33-0
MW: 120
Colorless, slightly viscous liquid (b.p. 543°F, 284°C).
Specific gravity/density: 1.2 at 30°C.
Vapor pressure: extremely low (0.0062 mm at 27°C)
Freezing point: 44°F, 7°C.
Solubilities: Miscible with water, miscible with benzene, toluene, acetone, and xylenes, and trichloroethylene.
Odor: odorless.
Nonreactive; slowly evolves sulfur dioxide (0.6-24 mg/hr) above 356°F, 180°C.



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APPENDIX F

METHOD FOR THE DETERMINATION OF CHEMICAL WARFARE (CW)
AGENTS IN NEUTRALIZATION MIXTURES USING A
GAS CHROMATOGRAPH/MASS SPECTROMETER (GC/MS)

ANALYTICAL CHEMISTRY TEAM METHOD 023

Mr. Thomas Rosso
Preparer

Mr. Mike Ellzy
Team Ldr, ACT

Mr. Dennis Johnson
QAC, Rsch & Tech 20 JUN 95

1. TITLE: **METHOD FOR THE DETERMINATION OF CHEMICAL WARFARE (CW) AGENTS IN NEUTRALIZATION MIXTURES USING A GAS CHROMATOGRAPH/ MASS SPECTROMETER (GC/MS)**

KEYWORDS: Bis(2-chloroethyl)sulfide (HD), Dichloro(2-chlorovinyl)arsine (Lewisite, L) , Bis(2-chloroethyl)ethylamine (HN-1), Non-Stockpile Chemical Materiel, 1,3-Dichloro-5,5-dimethylhydantoin, 1,2,4,5-Tetrachlorobenzene, Gas chromatography/mass spectrometer (GC/MS), Electron Ionization (EI)

2. CURRENT REVISION DATE: 13 June 1995

3. PREVIOUS REVISIONS: 17 February 1995
10 January 1995

4. OFFICE OF CONTACT:
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Edgewood Research, Development, and Engineering Center
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Aberdeen Proving Ground, MD 21010-5423

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5. APPLICATION:
The rapid response system (RRS) has been designed by non-stockpile chemical materials (NSCM) to neutralize chemical agents. Methods are being proposed for the rapid response system (RRS) for the neutralization of bis(2-chloroethyl)sulfide (HD), bis(2-chloroethyl)ethylamine (HN1) and dichloro(2-chloro-vinyl)arsine[Lewisite(L)]. An analysis method is necessary to certify that the neutralization reagent solution has converted the agent to a less toxic product. This analysis method requires a Hewlett-Packard 5989B MS Engine mass spectrometer with Chemstation data system. The quantitative analysis for this method will follow the equations for internal standardization using 1,2,4,5-tetrachlorobenzene.

Waste streams expected in the rapid response system (RRS) are as follows:

- 1 volume of neat HD treated with 20 volumes of 0.555M 1,3-Dichloro-5,5-dimethyl hydantoin (DCDMH) in 50/50 $CHCl_3$/tert-butanol with 3% water by volume.

- 1 volume of each 5-10% HD in $CHCl_3$, 5-10% HN1 in $CHCl_3$, and 5-10% L in $CHCl_3$ treated with 4 volumes of 0.555M 1,3-Dichloro-5,5-dimethyl hydantoin (DCDMH) in 50/50 $CHCl_3$/tert-butanol with 3% water by volume.

- 43% by weight HD and HN-1 on charcoal treated with excess 1,3-Dichloro-5,5-dimethylhydantoin in $CHCl_3$ combined with 43% by weight L with excess 1,3-Dichloro-5,5-dimethyl-hydantoin in $CHCl_3$/tert-butanol (50/50).

5.1 Tested Concentration Range:
Analyte concentration range will be tested at 50 mg/L for HD, HN-1, and L. The neutralent mixtures are reactive with the analytes of interest. A stable internal standard is added to the matrix in order to confirm the tested concentration level. These concentration levels are not necessarily the same as the instrument detection limit (IDL) because of the high neutralization reagent background and reactivity of the neutralent. It is also important for the testing levels to be sufficiently high to identify when the instrument is not functioning properly.

5.2 Sensitivity:
The calibration gas Perfluorotributylamine (PFTBA) ensures proper mass listing and tuning of the mass spectrometer. The GC/MS performance test will ensure proper IDL using the internal standard, 1,2,4,5-tetrachlorobenzene.

5.3 Detection Limit:
The instrument detection limit (IDL) of the GC/MS system is 1 mg/L with a signal to noise ratio of 10:1 or greater. The method detection limit (MDL) of the GC/MS is determined to be 10 mg/L for the internal standard in the neutralization solutions with a signal to noise ratio of 5:1 or greater. The method quantitation limit (MQL), or the minimum concentration of analyte that can be measured and reported, is 50 mg/L for HD, HN-1 and L.

5.4 Interferences:
No interferences with the peaks of interest were observed in any of the neutralization solutions. There are many other peaks in the chromatogram and they are of a percentage much greater than the peaks of interest (HD, L, HN-1). Retention times for the analytes of interest are identified in Table 1 for the HP MS Engine with electronic pressure control (EPP). The retention

times of the analytes is based the retention time of the internal standard, or relative retention time.

TABLE 1
ANALYTE RETENTION TIMES

ANALYTE	ACTUAL RETENTION (min.)	RELATIVE RETENTION (min.)
Tetrachlorobenzene	12.11 ±0.1	0
HN-1	9.37 ±0.1	2.73 ±0.1
HD	9.73 ±0.1	2.38 ±0.1
L	8.09 ±0.2	4.03 ±0.2

5.5 Analysis Rate:
The estimated number of samples analyzed by gas chromatography/ mass spectrometry (GC/MS) is 1 analysis per hour or 8 analysis per 8 hour working day. However, maintenance and cleaning of gas chromatograph may be necessary frequently due to degradation of GC components caused from arsenic compounds.

5.6 Validation:
The validation of this internal standard method involved testing of the stability of 1,2,4,5-tetrachlorobenzene in the neutralization matrices. In addition, this method was used to generate the data necessary to calculate the response factors for the analytes of interest.

6.0 SCIENTIFIC BASIS

6.1 Scientific Basis:
Chemical components of a mixture are separated on a chromatographic column and enter the mass spectrometer as a gas. The components are then collided with a high-energy electron beam. The energy produced from the collisions are so intense that the compounds form a variety of neutral and ionized fragments. The ionized fragments are sent toward the ampliplifier (detector) by charged electrodes and the signal generated is based on a mass to charge ratio.

6.2 Chemical Conversion Required:
No chemical conversion is necessary for the analysis of the agents of interest in the neutralization mixture.

7.0 APPARATUS

7.1 Instrumentation:
The Hewlett-Packard 5989B MS Engine chemstation system has been used for the determination of CW agents in neutralization mixtures. The specific parameters for the HP mass spectrometer is listed under the operating parameter section 9.4. A detailed quotation from Hewlett-Packard is included in the appendix for the HP 5989B mass spectrometer.

7.2 Hardware/Glassware/Miscellaneous Supplies:

(1) 10 µl syringes
(2) Vaporization injection port liners
(3) GC low bleed injection port septum (SIS 11mm #701-0041)
(4) DB-5 (95% dimethyl-5% diphenyl polysiloxane) fused silica column (Restek #10223)
(5) Split vent trap (charcoal)
(6) Ferrules
(7) Copper o-rings
(8) Spare ion source and ion volume
(9) Spare ion gauge
(10) Vacuum pump oil
(11) Screw cap glass vial (4mL)
(12) Calibrated pipets for 0.1 and 0.9 mL
(12) Pipets and pipet bulbs
(13) Adsorbent paper and towels
(14) Household bleach
(15) Safety glasses, lab coat and latex gloves

Sources:
SIS (Scientific Instrument Services, Inc. 1027 Old York Road, N.J. 08551-1039)
Restek (Restek Corporation, 110 Benner Circle, Bellefonte, PA 16823-8812)

7.3 Chemicals:

(1) 1,3-Dichloro-5,5-dimethylhydantoin (CAS# 77-48-5)
(2) Trichloromethane (CAS #67-66-3)
(3) Dichloromethane (CAS# 75-09-2)
(4) tert-Butyl alcohol (CAS# 75-65-0)
(5) 1,2,4,5-Tetrachlorobenzene (CAS# 95-94-3)
(6) Helium (99.9999% pure)

8. STANDARDS

a. Primary Standards:

(1) Bis(2-chloroethyl)sulfide (HD) (CAS# 505-60-2)
(2) Dichloro(2-chlorovinyl)arsine (L) (CAS# 541-25-3)
(3) Bis(2-chloroehtyl)ethylamine (HN-1)(CAS# 538-07-0)

b. Internal Standard:
(1) 1,2,4,5-Tetrachlorobenzene (CAS# 95-94-3)

Agents must be at least 95 mole percent pure and obtained from CASARM approved laboratory. The internal standard is purchased from Aldrich Chemical Co., Inc. (Milwaukee, Wisconsin).

9. PROCEDURE

9.1 Experimental Design:
A gas chromatograph/mass spectrometer (GC/MS) is used to determine composition of neutalization samples containing L, HD and HN-1. The neutralization mixtures are listed in section 5 (application). The MQL is based on an internal standard. An analyte of known concentration is combined with a standard of known concentration to generate a response factor for the specific analyte. This response factor (R_f) is used to generate quantitative analysis of the neutralization mixtures.

9.2 Safety:
Analyst may handle dilute chemical warfare agents and degradation products of chemical warfare agents. This analysis method is designed to minimize the possible hazard to the analyst from these agents and degradation products. Lab coats, latex gloves and safety glasses must be worn when handling the samples prior to injection into the GC/MS. A split vent trap must be attached to the Split/Splitless vent on the GC to trap any vented agent.

9.3 Instrument Calibration:
Daily checking for proper operation of the mass spectrometer is first accomplished by using the instrument's tuning and calibration macro and allowing the instrument to set electron multiplier voltage and emission current for optimum performance. Next, the internal standard will be used to ensure the instrument is operating at the defined method detection limit (50 mg/L).

9.4 Operating Parameters:
Table 2 is a list of parameters designed to be applied in the method section of the Hewlett-Packard chemstation.

TABLE 2
GC/MS PARAMETERS

-Acquisition mode	=	Full scan
-Ionization type	=	Electron Impact (EI)
-Injection	=	Split/Splitless
-Electron Voltage	=	Set during the autotune
-Emission Current	=	Set during the autotune
-Electron energy	=	70 eV

-Cycle time	=	≥ 1.0 /s
-Source Temp.	=	200 °C
-Quadrupole Temp.	=	100 °C
-Mass Gain	=	-8
-Mass Offs	=	33
-Repeller	=	7.00
-Low Mass	=	45 amu
-High Mass	=	525 amu
-Solvent delay	=	7 minutes
-Carrier gas	=	Helium
-Split ratio	=	49:1
-Split flow	=	30 ml/min.
-Split/Splitless delay	=	0.2 min.
-Injection flow	=	8.5psi @ 60 C
-Column flow	=	1.02 mL/min.
-Column velocity	=	36.8 cm/sec
-Injection Temp.	=	250 °C
-Column type	=	DB-5 (95% dimethyl- 5% diphenyl polysiloxane)
-Column length	=	30 meter
-Column diameter	=	0.25 mm ID
-Film thickness	=	0.25 µm
-Col. initial temp.	=	60 °C
-Col. initial hold	=	3 min.
-Col. program rate	=	10 °C/min.
-Col. final temp.	=	250 °C
-Col. final hold	=	5 min.
-Col. total time	=	27 min.

9.5 Test procedures for the determination of CW agents:

9.5.1

Examine the carrier gas, in this case Helium, to be sure it is flowing and in sufficient amount to run analysis.

9.5.2

The next step is EI (electron impact) instrumental calibration with calibration gas, PFTBA. This calibration needs to be done at least once a day or before a sequence of samples is analyzed. The HP MS Engine Chemstation system is equipped to tune the mass spectrometer automatically to achieve the following criteria:

m/z	69	=	100%
m/z	219	=	30-60%
m/z	414	=	1.4-4.0%
m/z	502	=	0.8-4.0%

The resolution of the m/z 69,70 and m/z 502,502 is tuned until a 10% valley and good peak shape are obtained. Isotope ratios are adjusted to match the true values of PFTBA as closely as possible; 70:69 = 1.1, 220:219 = 4.3, 415:414 = 9.0 and

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503:502 = 10.1. A variance of 20% is acceptable. Figure 1 is an example of the data generated from a mass spectrometer autotune. If the autotune does not obtain the specifications, then ensure the proper pressure is set for the calibration gas and repeat the calibration. If the second calibration does not obtain the proper specifications, refer to the Hewlett-Packard manual for proper troubleshooting guidence.

9.5.3

Prepare a stock solution of the internal standard in methylene chloride. Weigh out approximately 0.15 grams of 1,2,4,5-tetraclorobenzene in a tared 25 mL volumetric flask. Fill to the line with methylene chloride making the stock concentration 550 - 650 ppm.

9.5.4

The prepared internal standard will be used for a GC/MS performance test. The performance test will be used at least daily and also after every 5 sample injections or whenever performance appears poor. (The performance determination will be decided by the analyst) Ensure the split flow is set properly and the MS is prepared for the injection. Inject a 1 µl aliquot of the internal standard in the GC and start the MS collecting data. (NOTE: The solvent delay of 7 minutes will not allow the MS to collect data until after 7 minutes)

9.5.5

The following requirements must be met in order to accept the data from the GC/MS performance test and continue the sample sequence.

- ♦ Signal-to-noise ratio is > 5:1 in the total ion chromatogram (TIC) for the internal standard.
- ♦ The mass/charge values are correct in the EI spectra. The mass range to be printed should extend to molecular mass plus 60 amu to ensure the whole relevant mass range in each ionization mode. A reference EI spectra of 1,2,4,5-tetrachlorobenzene is included in the method.
- ♦ Deviation of relative ion abundances of main EI ions is < ±20% compared with the values in reference spectra.
- ♦ Retention time for the internal standard must be within ±0.1 mins.

If the criteria for accepting the performance test are not met, refer to the HP manuals for troubleshooting GC/MS.

9.5.6

A final quality control step will be the use of solvent blanks. A solvent blank is an injection with the solvent used in the samples ($CHCl_3$); it will be used to initiate a sequence; and it will be used between every sample injection. This step will eliminate the problem of "carry-over" and background interference from solvent. Table 3 is a typical sample sequence to be

followed during analysis.

TABLE 3
TYPICAL SAMPLE SEQUENCE

Autotune	PFTBA
Injection #1	GC/MS Perfomance Test
Injection #2	Solvent Blank
Injection #3	Sample #1
Injection #4	Solvent Blank
Injection #5	Sample #2
Injection #6	Solvent Blank
Injection #7	Sample #3
Injection #8	Solvent Blank
Injection #9	Sample #4
Injection #10	Solvent Blank
Injection #11	Sample #5
Injection #12	GC/MS Performance Test

9.5.6
Prepare a neutralization mixture for sample analysis. First, remove 0.9mL of the neutralization mixture and put it in a 4mL vial with plastic screw cap and Teflon cap liner. Second, remove 0.1mL of 1,2,4,5-tetrachlorobenzene (internal standard) from stock container. Combine internal standard with the neutralization mixture and replace the screw cap being sure to place the Teflon liner inside the cap. Mix the components by inverting the sample vial 10 times. The neutralization mixture is now prepared for injection into GC/MS. (NOTE: The internal standard in this method has been tested to be stable in the neutralization mixture for at least 72 hours with no degredation. Several samples may be prepared at once and then stored until it becomes time for injection into the GC/MS.)
9.5.7
The analysis of each sample consist of a 1 µl injection into th

GC/MS from the prepared neutralization mixture and internal standard using a 10 µl syringe. After injection into the GC, press start to begin the collection of the MS data. The syringe will be cleaned with an appropriate solvent and dried until analysis of the sample is complete.

9.5.8

After a sample injection, a solvent blank will always be injected. Fill a 4mL vial with 2mL of $CHCl_3$ and replace the cap. Remove 1ul of chloroform from from the 4mL $CHCl_3$ vial and inject into the GC. Collection of MS data will begin once the start button is depressed. If there is a significant amount of carryover, an additional solvent blank will be injected. If after three solvent blank injections the interference peaks do not recede, maintenence of the GC or MS may be necessary. (Refer to the HP manuals for information on troubleshooting GC/MS) If cleaning does not eliminate the peaks, note the peaks as interferences in a lab notebook and continue with sample sequence provided that the interferences do not affect the analysis of the agents.

9.6 Control Charts:

A logbook will be maintained and kept beside the instrument. The logbook will be a 3-ring binder containing the manufacturer, serial number, model number, and barcode of the instrument in use. Following this information, a separate section will contain reference spectra and mass lists, and relative retention time data in table 1. The logbook will also contain a record of the data sheet printed after every daily autotune i.e. figure 1. Next, a record of the GC/MS performance test with integration should be saved. Finally, the logbook will contain a list of the sample sequence runs from each day to include operators name, sample numbers, date, and the time each analysis was performed.

Standard quality control techniques used outside of this method are described in "Quality Assurance Project Plan for Analytical Chemistry Team Sample Analysis", QAPjP ACT-94-003.

9.7 System Control Methods:

Two control methods are used to test the operation and validity of the GC and MS:

A. Chromatographic GC/MS performance test

B. Perfluorotributylamine (PFTBA) calibration compound

For discussion of individual system performance capabilities, refer to instrumentation operations manual.

10.0 TREATMENT OF DATA
In addition to the data recorded from Section 9.6, hardcopies of the Total Ion Chromatogram, spectra, and agent identification information must be provided for each sample injection.

10.1 Concentration Determination:
Some concentration determinations may be desired for analytes of interest, however this method is qualitative. The following procedures are followed in this method for identification of analytes of interest and the quantitative analysis of the idetified analytes.

10.1.1 Criteria for Analyte Identification

The total ion chromatogram (TIC) is investigated at the relative retention times listed in table 1 for any peaks. The spectrum for these peaks should be analyzed for identification. The library search program on the Hewlett-Packard Chemstation is used to identify most peaks in the chromatogram. For analytes not available in the library from HP the following criteria must be followed for qualitative identification.

(1) All primary ions (ions greater that 10% of the most abundant ion) should be present in the sample and the reference spectrum, if the sample and the reference sample are at similar concentration levels.

(2) The relative intensities of the primary ions in the sample will not differ by more than 20% from the primary ions of the reference spectrum.

(3) The molecular ions present in the reference spectrum must be present int he sample spectrum, if reference and sample are of similar concentration.

10.1.2 Unknown concentration determination using the Internal Standardization Method

This method requires preparation before sample analysis begins. The internal standardization method utilizes a primary standard and an internal standard to calculate a response factor(R_f). Table 4 demonstrates the calculation for the R_f for HD.

TABLE 4
RESPONSE FACTOR DETERMINATION

Component	[ ]	[ ] Ratio Unk/IS	Area	Area Ratio Unk/IS
Unk **(HD)**	35.6ppm		775481	
		0.570		0.230
IS (tetrachloro-benzene)	62.4ppm		3365573	

R_f = Area Ratio/[ ] Ratio
R_f = 0.230/0.570
R_f = 0.404

TABLE 5
RESPONSE FACTORS FOR HD, HN-1 & L

Compound	Response Factor
HD	0.404
HN-1	0.484
L	0.172

Table 5 shows the response factors that will be used in this method. These response factors will be used in the following equation to determine the concentration of the analytes of interest.

$$[\ ]_{unknown} = (Area_{unknown} \times [\ ]_{standard}) \div (Area_{standard} \times \mathbf{R_f})$$

$[\ ]_{unknown}$ = Concentration of the unknown analyte **(HD,HN-1 or L)**
$Area_{unknown}$ = Peak area of the unknown analyte, integrated **by the** HP Chemstation
$[\ ]_{standard}$ = Concentration of the internal **standard**
$Area_{standard}$ = Peak area of the internal standard, integrated **by the** HP Chemstation
$\mathbf{R_f}$ = Response factor from table 4

Appendix F

REFERENCES

Grob, Robert L., Modern Practice of Gas Chromatography, John Wiley & Sons, 1977, pg. 199-201.

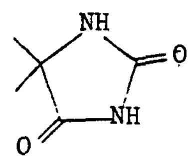
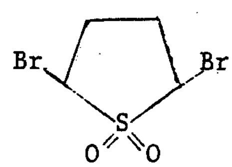
Blank

APPENDIX G

PRODUCT ANALYSIS (GC/MS) OF RED, BLUE, AND CHARCOAL PROCESS WASTESTREAMS

TABLES

TABLE G-1 Product Analysis (GC/MS/CI) of Brom55P-mediated Neutralization of CAIS Containing Neat HD (Initial Blue Process Chemistry)

Scan (sec)	MW	Compound	Area %[a,b]
79	122	$Cl_2CH_2CH_2SCH=CH_2$	2.6
113	166	$BrCH_2CH_2SCH=CH_2$	2.1
227	138	$ClCH_2CH_2S(O)CH=CH_2$	8.2
231	158	$ClCH_2CH_2SCH_2CH_2Cl$ (HD)[c]	0.2
339	216	$BrCH_2CH_2CH_2SCH_2CH_2Cl$ (or isomer)	6.0
381	128		N.Q.[d]
428	190	$ClCH_2CH_2S(O)CH_2CH_2Cl$ (HD sulfone)	1.5
444	174	$ClCH_2CH_2S(O)CH_2CH_2Cl$ (HD sulfoxide)	4.8
504	208	$ClCH_2CH_2CHBrS(O)CH_2CH_2$ (or isomer)	1.5
538	252	$ClCH_2CH_2CHBrS(O)CH_2CH_2Cl$ (or isomer)	11.0
553	276		N.Q.
567	252	$ClCH_2CHBrS(O)CH_2CH_2Cl$ (or isomer)	62.0

a-Area % does not include solvent and oxidant areas; calculated from the Total Ion Chromatogram (TIC) of the mass spectrometer
b- Area % is semi-quantitative, intent is to show the percent of a peak in comparison to other peaks in the chromatogram. Peaks less than 0.1% of the TIC are not quantitated.
c-Method Quantitation Limits (MQL) for agent is 100 ppm via GC/MS/CI analysis.
d-N.Q. Not Quantitated

KEY COMPONENTS SYNOPSIS

Component	Area %
HD	0.2
HD Sulfone	1.5
HD Sulfoxide	4.8
Sulfoxides	82.7
Sulfides	10.7
Unknown(s)	-
Total	99.9

TABLE G-2 Product Analysis (GC/MS/CI) of DCDMH-mediated Neutralization of CAIS Containing Neat HD (Modified Blue Process Chemistry)

Scan (sec)	MW	Compound	Area[a,b] %
58	110	Unknown, contains 1 chlorine	2.6
74	110	Unknown, contains 1 chlorine	2.9
86	122	$ClCH_2CH_2SCH=CH_2$	8.0
103	124	trichlorobutene	0.7
114	106	Unknown, no chlorine	
116	136	$CH_2=CHS(O)CH=CHCl$	1.5
124	120	isomer of 1,4 dithiane	1.9
146	156	isomer of $ClCH_2CH_2SCH=CHCl$	7.7
157	120	1,4 dithiane	0.1
168	118	$CH_2=CHS(O_2)CH_2CH_2$ (divinyl sulfone)	0.3
195		Unknown	0.3
223	138	$CH_2=CHS(O)CH_2CH_2Cl$	
	156	$CH_2ClCH_2SCH=CHCl$ isomer	2.3
265	172	$ClCH_2CH_2S(O)CH=CHCl$ isomer	55.0
278	172	$ClCH_2CH_2S(O)CH=CHCl$ isomer	1.4
332	206	$ClCH_2CHClS(O)CH=CHCl$ isomer	0.8
386	206	$ClCH_2CHClS(O)CH=CHCl$ isomer	0.8
409	220	Unknown, contains 3 chlorines	1.1
414	190	$ClCH_2CH_2S(O)_2CH_2CH_2Cl$ (HD sulfone)	1.2
464	208	$ClCH_2CHClS(O)CH_2CH_2Cl$ isomer	1.0
496	208	$ClCH_2CHClS(O)CH_2CH_2Cl$ isomer	8.3
509	242	$ClCH_2CHClS(O)CHClCH_2Cl$ isomer	0.8
543	242	$ClCH_2CHClS(O)CHClCH_2Cl$ isomer	1.2

a-Area % does not include solvent and oxidant areas; area % calculated from the Total Ion Chromatogram (TIC) of the mass spectrometer.
b-Area % is semi-quantitative, the intent is to show the percent of the peak in comparison to other peaks in the chromatogram. Peaks less than 0.1% of the TIC are not quantitated.

KEY COMPONENTS SYNOPSIS

Component	Area %
HD	(-)
HD Sulfone	1.2
Divinyl sulfone	0.3
HD Sulfoxide	(-)
Sulfoxides	71.2
Sulfides	16.8
Unknown(s)	7.7
Other	2.7
Total	99.9

TABLE G-3 Product Analysis (GC/MS/EI) of m-CPBA-mediated Neutralization of CAIS Containing HD, HN-1, and L in Chloroform (Initial Red Process Chemistry)[a]

Scan (sec)	Compound	Area[b,c] %
54	dichlorobutene	2.1
58	dichlorobutene, chlorobenzene	3.8
77	chlorinated unknown	2.4
95	trichlorobutene	7.3
180	chlorinated unknown	0.8
240	bis(2-chloroethyl)ethylamine (HN-1)[d]	10.9
261	2-chloroethyl vinyl sulfone	0.4
267	2-chloroethyl- 2-chloroethyl sulfoxide (HD sulfoxide)	0.4
307	unknown	1.0
327	dichlorethyl dichloroethyl sulfoxide	0.8
419	m-chlorobenzoic acid, t-butyl ester	30.9
441	bis(2-chloroethyl) sulfone (HD sulfone)	20.9
487	m-chlorobenzoic acid	N.Q.[e]
499	chloroethyl dichloroethyl sulfoxide	1.2
530	chloroethyl dichloroethyl sulfoxide isomer	14.3
583	chlorobenzoic acid isomer	2.8
665	Unknown	0.2
821	Unknown	2.0

a-Composition analysis conducted via GC-MS Electron Ionization (EI) mode which does not provide Molecular Weight (MW) information.
b-The area % does not include solvent and oxidant areas. Area percent calculated from the Total Ion Chromatogram (TIC) of the mass spectrometer.
c-Area % is semi-quantitative, intent is to show the percent of the peak in comparison to other peaks in the chromatogram. Peaks less than 0.1% of the TIC are not quantitated.
d-Method Quantitation Limit (MQL) for agent is 50 ppm for GC/MS/CI methodology.
e-N.Q.=Not Quantitated

KEY COMPONENTS SYNOPSIS

Component	Area %
HD	(-)
HD Sulfone	20.9
HD Sulfone analog	0.4
HD Sulfoxide	0.4
Sulfoxides	16.3
Sulfides	(-)
Unknown(s)	6.4
HN-1	10.9
L	(-)
Other	46.9
Total	102.2

TABLE G-4 Product Analysis (GC/MS/CI) of DCDMH-mediated Neutralization of CAIS Containing HD, HN-3 and L in Chloroform (Modified Red Process Chemistry)[a,b]

Scan (sec)	MW	Compound	Area[c,d] %
72	126	dichlorobutane	1.2
82	124	dichlorobutene	2.0
99	160	trichlorobutane	5.9
103	160	trichlorobutane	5.1
121	136	unknown	0.6
135	162	unknown, contains 1 chlorine	1.5
139	194	tetrachlorobutane	2.9
153	194	tetrachlorobutane	1.0
158	206	$Cl_2AsCH=CHCl$ [Lewisite (L_1)]	0.7
165	194	tetrachlorobutane	6.2
199	192	tetrachlorobutene	0.7
215	194	tetrachlorobutane	5.1
219	172	unknown, contains 1 clorine	1.1
256	172	$ClCH_2CH_2S(O)CH=CHCl$	8.2
283	192	tetrachlorobutane	0.5
303	232	$ClAs(CH=CHCl)_2$ [Lewisite (L_2)]	1.4
327	206	$ClCH_2CHClS(O)CH=CHCl$	1.3
396	203	$N(CH_2CH_2Cl)_3$ (HN-3)	4.0
412	190	$ClCH_2CH_2S(O)_2CH_2CH_2Cl$	0.5
510	[242]		[41.5]
544	[242]	$ClCH_2HClS(O)CHClCH_2Cl$ (isomers)	[8.5]

a-HN-3 represented the nitrogen mustard in the chemical neutralization reactor in place of HN-1.
b-Method Quantitation Limit (MQL) of agent is 100 ppm with GC/MS/CI.
c-Area % does not include solvent and oxidant areas, calculated from the Total Ion Chromatogram (TIC) of the mass spectrometer.
d-Area % is semi-quantitative, intent is to show the percent of the peak in comparison to other peaks in the chromatogram. Peaks less than 0.1% of the TIC are not quantitated.

KEY COMPONENTS SYNOPSIS

Component	Area %
HD	(-)
HD Sulfone	0.5
HD Sulfoxide	(-)
HN-3	4.0
L_1	0.7
L_2	1.4
Sulfoxides	59.5
Sulfides	(-)
Unknown(s)	3.2
Other	30.0
Total	99.9

TABLE G-5 Product Analysis (GC/MS/CI) of DCDMH-mediated Neutralization of CAIS Containing HD, HN-1, and L on Charcoal (Modified "Charcoal Process" Chemistry)

Scan (sec)	MW	Compound	Area[a,b,c] %
27	74	t-butanol	7.4
30	118	chloroform	15.3
37	164	$Cl_3CCH(OH)_2$	2.3
39	216	unknown, 2 or 3 chlorines	3.6
45	132	trichlorobutane	1.6
52	164	unknown, 4 chlorines	0.1
59	-	unknown	0.4
62	-	Unknown	4.7
74	124	dichlorobutene	0.3
81	[124 130]	dichlorobutene Unknown, 2 chlorines	[1.5]
86	158	trichloroethane	0.2
100	124	dichlorobutene	2.6
102	158	trichlorobutene	0.9
105	158	trichlorobutene	0.9
110	[164 208]	unknown, 4 chlorines	[4.8]
113	174	unknown, 3 chlorines	0.1
141	158	trichlorobutene	4.4
146	194	tetrachlorobutane	0.2
152	208	unknown, 4 chlorines	0.2
160	158	trichlorobutene	0.2
163	198	unknown, 5 chlorines	3.3
173	158	trichlorobutene	4.1
176	228	pentachlorobutane	2.8
190	216	unknown, 3 chlorines	0.1
207	176	unknown, 3 chlorines	0.4
224	194	tetrachlorobutane	2.5
228	162	unknown, 3 or 4 chlorines	0.2
235	192	tetrachlorobutane	0.1
239	262	hexachlorobutene	0.4
293	192	tetrachlorobutane	1.6
306	224	unknown, 4 chlorines	0.2
336	218	unknown, 3 chlorines	1.0
340	218	unknown, 3 chlorines	0.2
347	218	unknown, 3 chlorines	0.6
368	292	unknown, 6 chlorines	0.5
390	274	unknown, 5 or 6 chlorines	0.2
402	250	unknown, 4 chlorines	0.3
429	236	unknown, 4 chlorines	2.0
438	236	unknown, 4 chlorines	0.3
470	310	unknown, 5 or 6 chlorines	0.7
492	272	unknown, 5 chlorines	0.5

TABLE G-5 (continued)

Scan (sec)	MW	Compound	Area[a,b,c] %
497	**272**	**unknown, 5 chlorines**	**0.8**
501	**272**	**unknown, 5 chlorines**	**0.4**
509	**272**	**unknown, 5 chlorines**	**0.2**
561	**296**	**heptachlorobutane**	**1.5**
610	**258**	**unknown, 3 chlorines**	**0.6**
642	**258**	**unknown, 3 chlorines**	**0.1**
650	**292**	**unknown, 4 or 5 chlorines**	**0.1**
685	**292**	**unknown, 4 or 5 chlorines**	**0.06**
709	**292**	**unknown, 4 or 5 chlorines**	**0.04**
400-550	**128**	**unknown**	**22.8**

a-Area % calculated from the Total Ion Chromatogram (TIC) of the mass spectrometer.
b-Area % is semi-quantitative; intent is to show the percent of the peak in comparison to other peaks in the chromatogram.
c-Method Quantitation Limit (MQL) for agent is 50 ppm.

Key Components Synopsis

Component	Area %
$CHCl_3$/t-Butanol	22.7
HD	(-)[a]
HD sulfone	(-)
HD sulfoxide	(-)
HN-1	(-)
HN-1 oxide	(-)
L	(-)
Sulfides	(-)
Sulfoxides	(-)
Chlorinated alkanes	10.5
Chlorinated alkenes	14.5
Other	2.3
Unknown(s)	50.3
Total	100.2

[a] (-) Not Detected

Blank

APPENDIX H

DERMAL IRRITATION RESPONSE: 4-HR OCCLUDED EXPOSURE TO INITIAL "BLUE PROCESS" REAGENTS AND WASTESTREAM

TABLES

TABLE H-1 Irritation Response Data: 4-hour Occluded Exposure to Sulfolane

Dermal Irritation Testing
(4-hour Occluded)

OBSERVATION PERIODS

Animal #	Ear Tag	Sex	Weight (kg)	Dose (mL)	Time Given	Time Removed	4 Hr*	24 Hr	48 hr	72 Hr	7 Day	14 Day		Final Weight (kg)
5	1167	♂	3.00	0.5	0948	1349	Ery None Ed None	Ery None Ed None	Ery None Ed None	Ery +1 Ed None	Ery +4 Ed None	Ery +4 Ed None		3.24
6	1199	♂	3.10	0.5	0949	1350	Ery None Ed None	Ery None Ed None	Ery None Ed None	Ery None Ed None	Ery None Ed None	Ery None Ed None		3.33
7	077	♀	2.85	0.5	0949.5	1351	Ery None Ed None	Ery None Ed None	Ery None Ed None	Ery None Ed None	Ery None Ed None	Ery None Ed None		2.97
8	074	♀	2.69	0.5	0950	1352	Ery None Ed None	Ery None Ed None	Ery None Ed None	Ery None Ed None	Ery None Ed None	Ery None Ed None		3.02

*-denotes contact time of 4 hours with initial observations recorded 30-60 minutes after removal of test material.

Date: 21 Mar 95 **Compound Identification:** Sulfolane **Physical State:** Liquid

Type of Test: Skin Irritation **Animal Strain/Species:** NZW Rabbit **pH:** N/A

Operators: K. Cameron, J. Manthei, D. Heithamp, D. Bona

Notes and Observations:

TABLE H-2 Irritation Response Data: 4-hour Occluded Exposure to Brom55P/Sulfolane

Dermal Irritation Testing
(4-hr Occluded Exposure)

Animal #	Ear Tag	Sex	Weight (kg)	Dose (mL)	Time Given	Time Removed	OBSERVATION PERIODS 4 Hr*	24 Hr	48 hr	72 Hr	7 Day	14 Day	Final Weight (kg)
9	1198	♂	2.65	0.5	0952.5	1303	Ery +1 Ed None	Ery +2 Ed +1	Ery +2 Ed +1	Ery +2 Ed None	Ery +2 Ed None	Ery +2 Ed +1	2.86
10	1162	♂	2.92	0.5	0954	1304	Ery +1 Ed None	Ery +2 Ed None	Ery +2 Ed +1	Ery None Ed None	Ery None Ed None	Ery None Ed None	3.29
11	073	♀	2.83	0.5	0954.5	1305	Ery +1 Ed None	Er +3 Ed +3	Ery +4 Ed +4	Ery +4 Ed +4	Ery +4 Ed +4	Ery +4 Ed +1	3.05
12	059	♀	3.00	0.5	0955	1306	Ery +1 Ed None	Ery +3 Ed +3	Ery +4 Ed +4	Ery +4 Ed +4	Ery +4 Ed +4	Ery +4 Ed +1	3.41

*-denotes contact time of 4 hours with initial observations recorded 30-60 minutes after removal of test material.

Date: 21 March 95

Compound Identification: Oxidizer/Solvent Mixture (Brom55P/Sulfolane)

Physical State: Liquid

Type of Test: Skin Irritation

Animal Strain/Species: NZW Rabbit

pH: N/A

Operators: K. Cameron, J. Manthei, D. Heithamp, D. Bona

Notes and Observations:

TABLE H-3 Irritation Response Data: 4-hr Occluded Exposure to Initial "Blue Process Chemistry" Wastestream

Dermal Irritation Testing
(4-hr Occluded Exposure)

OBSERVATION PERIODS

Animal #	Ear Tag	Sex	Weight (kg)	Dose (mL)	Time Given	Time Removed	4 Hr*	24 Hr	48 hr	72 Hr	7 Day	14 Day	Final Weight (kg)
13	1196	♂	2.84	0.5	1004	1405	Ery +2 Ed None	Ery +2 Ed +3	Ery +3 Ed +3	Ery +4 Ed +2	Ery +4 Ed +4	Ery +4 Ed +4	3.13
14	1175	♂	2.85	0.5	1005	1406	Ery +2 Ed None	Ery +2 Ed +3	Ery +3 Ed +2	Ery +4 Ed +2	Ery +4 Ed None	Ery +2 Ed +1	3.08
15	063	♀	2.81	0.5	1005.5	1407.5	Ery +2 Ed None	Er +2 Ed +2	Ery +2 Ed +2	Ery +1 Ed +1	Ery +4 Ed None	Ery None Ed None	3.00
16	097	♀	2.90	0.5	1006.5	1408	Ery +1 Ed None	Ery +2 Ed +3	Ery +2 Ed +3	Ery +4 Ed +4	Ery +4 Ed +4	Ery +4 Ed +2	3.21

*-denotes contact time of 4 hours with initial observations recorded 30-60 minutes after removal of test material.

Date: 21 March 95 **Compound Identification:** Wastestream Mixture (Brom55P mediated) **Physical State:** Liquid

Type of Test: Skin Irritation **Animal Strain/Species:** NZW Rabbit **pH:** N/A

Operators: K. Cameron, J. Manthei, D. Heithamp, D. Bona

Notes and Observations:

APPENDIX I

IRRITATION RESPONSE DATA: 4-HR OCCLUDED EXPOSURE TO MODIFIED "BLUE PROCESS" WASTESTREAM

TABLE I **Irritation Response Data: 4-hr Occluded Exposure to Modified "Blue Process Chemistry" Wastestream**

Dermal Irritation Testing

(4-hr Occluded Exposure)

OBSERVATION PERIODS

Animal #	Ear Tag	Sex	Weight (kg)	Dose (mL)	Time Given	Time Removed	4 Hr*	24 Hr	48 hr	72 Hr	7 Day	14 Day	Final Weight (kg)
15	401	♂	2.77	0.5	0926.5	1334	Ery +2 Ed +2	Ery +2 Ed +2	Ery +3 Ed +2	Ery +4 Ed +2	Ery +4 Ed None	Ery +4 Ed None	2.96
6	402	♂	3.20	0.5	0927	1335	Ery +2 Ed +2	Ery +3 Ed +2	Ery +3 Ed +2	Ery +4 Ed +2	Ery +4 Ed None	Ery +4 Ed None	3.35
17	389	♂	3.03	0.5	0927.5	1337	Ery +2 Ed +2	Ery +2 Ed +2	Ery +4 Ed +2	Ery +4 Ed +2	Ery +4 Ed None	Ery +3 Ed None	3.14
73	1738	♀	2.61	0.5	0928.5	1338	Ery +1 Ed +3	Ery +2 Ed +2	Ery +3 Ed +2	Ery +4 Ed +2	Ery +4 Ed None	Ery +2 Ed None	2.87
78	1742	♀	2.52	0.5	0929	1339	Ery +1 Ed +3	Ery +3 Ed +2	Ery +4 Ed +2	Ery +4 Ed +2	Ery +4 Ed None	Ery +2 Ed None	2.78
67	1730	♀	2.30	0.5	0929.5	1340	Ery +2 Ed +2	Ery +3 Ed +2	Ery +4 Ed +2	Ery +4 Ed +2	Ery +4 Ed None	Ery +3 Ed None	2.66

*-denotes contact time of 4 hours with initial observations recorded 30-60 minutes after removal of test material.

Date: 10 August 95 **Compound Identification:** Wastestream Mixture (DCDMH as oxidizer) **Physical State**: Liquid

Type of Test: Skin Irritation **Animal Strain/Species:** NZW Rabbit **pH:** N/A

Operators: K. Cameron, J. Manthei, D. Heithamp, D. Bona

Notes and Observations:

APPENDIX J

DERMAL IRRITATION RESPONSE DATA: OCCLUDED EXPOSURE TO INITIAL "RED PROCESS" REAGENTS AND WASTESTREAM

TABLES

Irritation Response Data: 4-Hr Occluded Exposure to m-CPBA/$CHCl_3$

Irritation Response Data: 4-Hr Occluded Exposure to Initial "Red Process" Wastestream

TABLE J-1 Irritation Response Data: 4-hour Occluded Exposure to m-Chloroperoxybenzoic acid (m-CPBA) in chloroform/t-butanol

Dermal Irritation Testing
(4-hour Occluded Exposure)

OBSERVATION PERIODS

Animal #	Ear Tag	Sex	Weight (kg)	Dose (mL)	Time Given	Time Removed	4 Hr*	24 Hr	48 hr	72 Hr	7 Day	14 Day	Final Weight (kg)
1	1678	♂	2.34	0.5	1014	1414	Ery +2 Ed +4	Ery +2 Ed +4	Ery +4 Ed +4	Ery +4 Ed +4	Ery +3 Ed - +4	Ery - +2** Ed - None	2.65
2	1679	♂	2.40	0.5	1015	1415	Ery +2 Ed +4	Ery +3 Ed +4	Ery +4 Ed +4	Ery +4 Ed +4	Ery +2 Ed - +4	Ery - +2 Ed - +4	2.68
17	1689	♂	2.51	0.5	1015.5	1416	Ery +2 Ed +4	Ery +4 Ed +4	Ery +4 Ed +4	Ery +4 Ed +4	Ery +4 Ed - +4	Ery - +3 Ed - +4	2.80
36	1652	♀	2.40	0.5	1016	1417	Ery +2 Ed +4	Ery +4 Ed +4	Ery +4 Ed +4	Ery +4 Ed +4	Ery +3 Ed - +4	Ery +3 Ed +4	2.71
37	1657	♀	2.36	0.5	1017	1418	Ery +2 Ed +4	Ery +4 Ed +4	Ery +4 Ed +4	Ery +4 Ed +4	Ery +4 Ed - +4	Ery - +4 Ed - +4	2.59
38	1671	♀	2.50	0.5	1017.5	1419	Ery +2 Ed +4	Ery +4 Ed +4	Ery +4 Ed +4	Ery +4 Ed +4	Ery +4 Ed - +4	Ery- +3 Ed - +4	2.67

*-denotes contact time of 4 hours with initial observations recorded 30-60 minutes after removal of test material.

Date: 19July 95 **Compound Identification:** m-CPBA/chloroform/t-butanol **Physical State:** Liquid

Type of Test: Skin Irritation **Animal Strain/Species:** NZW Rabbit **pH:** N/A

Operators: K. Cameron, J. Manthei, D. Heithamp, D. Bona

Notes and Observations: ** 14 day observations revealed a reversal (healing) of erythema. The eschar was beginning to peel off revealing scar tissue beneath

TABLE J-2 Irritation Exposure Data: 24-hour Occluded Exposure to Initial "Red Process Chemistry" Wastestream

Dermal Irritation Testing
(24-hour Occluded Exposure)

Animal #	Ear Tag	Sex	Weight (kg)	Dose (mL)	Time Given	Time Removed	OBSERVATION PERIODS 24 Hr	48 Hr	72 hr	7 Day	14 Day	Final Weight (kg)
19	1171	♂	3.06	0.5	0921	0921	Ery +3 Ed +4	Ery +4 Ed +4	Ery +4 Ery +4	Ery +4 Ed +3	Ery +4 Ed None	3.08
20	1189	♂	2.99	0.5	0922	0923	Ery +4 Ed +4	Ery +4 Ed +4	Ery +4 Ed +4	Ery +4 Ed +2	Ery +4 Ed None	3.10
21	1182	♂	3.07	0.5	0923	0925	Ery +4 Ed +4	Er +4 Ed +4	Ery +4 Ed +4	Ery +4 Ed +2	Ery +4 Ed None	3.06
22	1185	♂	3.26	0.5	0924	0927	Ery +3 Ed +4	Ery +4 Ed +4	Ery +4 Ed +4	Ery +4 Ed +2	Ery +4 Ed None	3.25
23	1183	♂	3.38	0.5	0924.5	0929	Ery +3 Ed +4	Ery +4 Ed +4	Ery +4 Ed +4	Ery +4 Ed +2	Ery +4 Ed None	3.51
24	076	♀	3.05	0.5	0925.5	0931	Ery +3 Ed +4	Ery +4 Ed +4	Ery +4 Ed +4	Ery +4 Ed +2	Ery +4 Ed +1	3.13
25	084	♀	3.21	0.5	0926.5	0933	Ery +3 Ed +4	Ery +4 Ed +4	Ery +4 Ed +4	Ery +4 Ed +2	Ery +4 Ed None	3.28
26	088	♀	3.20	0.5	0927	0935	Ery +4 Ed +4	Ery +4 Ed +4	Ery +4 Ed +4	Ery +4 Ed +2	Ery +4 Ed +1	3.21
27	087	♀	3.39	0.5	0927.5	0938	Ery +4 Ed +4	Ery +4 Ed +4	Ery +4 Ed +4	Ery +4 Ed +2	Ery +4 Ed +1	3.39
28	071	♀	3.08	0.5	0928	0940	Ery +4 Ed +4	Ery +4 Ed +4	Ery +4 Ed +4	Ery +4 Ed +2	Ery +4 Ed None	3.06

Date: 18 April 95

Compound Identification: Initial Red Process Wastestream (m-CPBA as oxidizer)

Physical State: Liquid

Type of Test: Skin Irritation

Animal Strain/Species: NZW Rabbit

pH: N/A

Operators: K. Cameron, J. Manthei, D. Heithamp, D. Bona

Notes and Observations:

Blank

APPENDIX K

DERMAL IRRITATION RESPONSE DATA: 4-HR OCCLUDED EXPOSURE TO MODIFIED "RED PROCESS" REAGENTS AND WASTESTREAM

TABLES

TABLE K-1 Irritation Response Data: 4-hour Occluded Exposure to Chloroform

Dermal Irritation Testing
(4-hour Occluded Exposure)

OBSERVATION PERIODS

Animal #	Ear Tag	Sex	Weight (kg)	Dose (mL)	Time Given	Time Removed	4 Hr*	24 Hr	48 hr	72 Hr	7 Day	14 Day	Final Weight (kg)
1	179	♂	2.74	0.5	1047	1447	Ery +1 Ed +2	Ery +2 Ed +4	Ery +2 Ed +3	Ery +1 Ed None	Ery None Ed None	Ery None Ed None	2.28
2	164	♂	2.95	0.5	1048	1448	Ery +1 Ed +2	Ery +2 Ed +3	Ery +2 Ed +3	Ery +2 Ed None	Ery None Ed None	Ery None Ed None	2.50
3	176	♂	2.86	0.5	1048.5	1448.5	Ery +1 Ed +2	Er +1 Ed +3	Ery +2 Ed +3	Ery +1 Ed None	Ery None Ed None	Ery None Ed None	3.02
6	1458	♀	2.66	0.5	1049	1450	Ery +1 Ed +2	Ery +2 Ed +2	Ery +2 Ed +2	Ery +1 Ed None	Ery None Ed None	Ery None Ed None	2.91
7	1471	♀	2.85	0.5	1049.5	1451	Ery +1 Ed +2	Ery +2 Ed +3	Ery +2 Ed +2	Ery +2 Ed None	Ery None Ed None	Ery None Ed None	3.09
8	1459	♀	2.84	0.5	1050.5	1452	Ery +1 Ed +2	Ery +1 Ed +2	Ery +2 Ed +2	Ery +2 Ed None	Ery None Ed None	Ery None Ed None	3.15

*-denotes contact time of 4 hours with initial observations recorded 30-60 minutes after removal of test material.

Date: 24 May 95 **Compound Identification:** Chloroform Component **Physical State:** Liquid

Type of Test: Skin Irritation **Animal Strain/Species:** NZW Rabbit **pH:** N/A

Operators: K. Cameron, J. Manthei, D. Heithamp, D. Bona

Notes and Observations:

TABLE K-2 Irritation Response Data: 4-hour Occluded Exposure to tertiary-Butanol

Dermal Irritation Testing
(4-hour Occluded Exposure)

Animal #	Ear Tag	Sex	Weight (kg)	Dose (mL)	Time Given	Time Removed	OBSERVATION PERIODS 4 Hr*	24 Hr	48 hr	72 Hr	7 Day	14 Day	Final Weight (kg)
4	161	♂	2.45	0.5	1051.5	1453	Ery None Ed None	Ery None Ed None	Ery None Ed None	Ery None Ed None	Ery None Ed None	Ery None Ed None	2.65
5	165	♂	2.83	0.5	1052	1454	Ery None Ed None	Ery None Ed None	Ery None Ed None	Ery None Ed None	Ery None Ed None	Ery None Ed None	3.08
11	166	♂	2.85	0.5	1052.5	1455	Ery None Ed None	Er None Ed None	Ery None Ed None	Ery None Ed None	Ery None Ed None	Ery None Ed None	3.04
9	1455	♀	2.71	0.5	1053	1456	Ery None Ed None	Ery None Ed None	Ery None Ed None	Ery None Ed None	Ery None Ed None	Ery None Ed None	2.87
10	1469	♀	2.60	0.5	1053.5	1457	Ery None Ed None	Ery None Ed None	Ery None Ed None	Ery None Ed None	Ery None Ed None	Ery None Ed None	2.91
16	1457	♀	2.72	0.5	1054	1458	Ery None Ed None	Ery None Ed None	Ery None Ed None	Ery None Ed None	Ery None Ed None	Ery None Ed None	3.02

*-denotes contact time of 4 hours with initial observations recorded 30-60 minutes after removal of test material.

Date: 24 May 95 **Compound Identification:** t-Butanol **Physical State:** Liquid

Type of Test: Skin Irritation **Animal Strain/Species:** NZW Rabbit **pH:** N/A

Operators: K. Cameron, J. Manthei, D. Heithamp, D. Bona

Notes and Observations:

TABLE K-3 Irritation Response Data: 4-hr Occluded Exposure to Chloroform/t-Butanol (50:50 mixture)

Dermal Irritation Testing
(4-hr Occluded Exposure)

OBSERVATION PERIODS

Animal #	Ear Tag	Sex	Weight (kg)	Dose (mL)	Time Given	Time Removed	4 Hr*	24 Hr	48 hr	72 Hr	7 Day	14 Day	Final Weight (kg)
12	170	♂	2.54	0.5	1056	1459	Ery +1 Ed None	Ery +1 Ed +1	Ery +2 Ed +3	Ery +1 Ed None	Ery None Ed None	Ery None Ed None	2.73
13	182	♂	2.54	0.5	1056.5	1500	Ery +1 Ed +1	Ery +2 Ed +2	Ery +2 Ed +3	Ery +1 Ed None	Ery None Ed None	Ery None Ed None	2.76
14	180	♂	2.75	0.5	1057.5	1501	Ery +1 Ed +1	Er +1 Ed +3	Ery +3 Ed +3	Ery +1 Ed None	Ery None Ed None	Ery None Ed None	2.99
17	1470	♀	2.95	0.5	1058	1502	Ery +2 Ed +1	Ery +2 Ed +2	Ery +2 Ed +2	Ery +2 Ed +1	Ery None Ed None	Ery None Ed None	2.97
18	1463	♀	2.85	0.5	1058.5	1513	Ery +1 Ed +1	Ery +2 Ed +1	Ery +2 Ed +2	Ery +2 Ed +2	Ery None Ed None	Ery None Ed None	3.01
19	1468	♀	2.97	0.5	1059	1504	Ery +1 Ed +2	Ery +2 Ed +2	Ery +2 Ed +2	Ery +2 Ed +1	Ery None Ed None	Ery None Ed None	3.23

*-denotes contact time of 4 hours with initial observations recorded 30-60 minutes after removal of test material.

Date: 24 May 95 **Compound Identification:** Co-Solvent System (50:50 Chloroform:t-butanol) **Physical State**: Liquid

Type of Test: Skin Irritation **Animal Strain/Species:** NZW Rabbit **pH:** N/A

Operators: K. Cameron, J. Manthei, D. Heithamp, D. Bona

Notes and Observations:

TABLE K-4 Irritation Response Data: 4-hour Occluded Exposure to DCDMH/Chloroform/tertiary-Butyl alcohol Mixture

Dermal Irritation Testing
(4-hour Occluded Exposure)

OBSERVATION PERIODS

Animal #	Ear Tag	Sex	Weight (kg)	Dose (mL)	Time Given	Time Removed	4 Hr*	24 Hr	48 hr	72 Hr	7 Day	14 Day	Final Weight (kg)
25	377	♂	3.00	0.5	0921.5	1326	Ery +4 Ed + 4	Ery +4 Ed +3	Ery +4 Ed +3	Ery +4 Ed +3	Ery +4 Ed - +3	Ery +4 Ed - +3	3.17
32	392	♂	2.85	0.5	0922.5	1327	Ery +4 Ed +4	Ery +4 Ed +3	Ery +4 Ed +3	Ery +4 Ed +3	Ery +4 Ed - +3	Ery +4 Ed - +3	2.81
33	391	♂	3.16	0.5	0923	1329	Ery +4 Ed +4	Ery +4 Ed +3	Ery +4 Ed +4	Ery +4 Ed +2	Ery +4 Ed - +3	Ery +3 Ed - +3	2.97
70	1733	♀	2.69	0.5	0923.5	1330	Ery +4 Ed +4	Ery +4 Ed +3	Ery +4 Ed +3	Ery +4 Ed +3	Ery +4 Ed - +3	Ery +4 Ed - +2	3.38
69	1737	♀	2.42	0.5	0924	1331	Ery +4 Ed +4	Ery +4 Ed +3	Ery +4 Ed +3	Ery +4 Ed +2	Ery +4 Ed - +3	Ery +4 Ed - +2	2.67
77	1732	♀	2.70	0.5	0925	1332.5	Ery +4 Ed +4	Ery +4 Ed +3	Ery +4 Ed +4	Ery +4 Ed +3	Ery +4 Ed - +3	Ery +4 Ed - +3	2.99

*-denotes contact time of 4 hours with initial observations recorded 30-60 minutes after removal of test material.

Date: 10 Aug 95 **Compound Identification:** DCDMH Oxidizer/Co-solvent Mixture (DCDMH/Chloroform/t-Butyl alcohol) **Physical State:** Liquid

Type of Test: Skin Irritation **Animal Strain/Species:** NZW Rabbit **pH:** N/A

Operators: K. Cameron, J. Manthei, D. Heithamp, D. Bona

Notes and Observations: Skin "draws up" immediately upon dosing. At 7-days, edema still present, although mild. Eschar dry, cracked and peeling. Some scar tissue evident beneath the eschar. Necrotic tissue also evident. Deep subcutaneous tissue damage with blanched areas evident indicating damage to the muscle layer.

TABLE K-4 (con't) Irritation Response Data: 4-hour Occluded Exposure to DCDMH/Chloroform/tertiary-Butyl alcohol Mixture

Dermal Irritation Testing
(4-hour Occluded Exposure)

OBSERVATION PERIODS

Animal #	Ear Tag	Sex	Weight (kg)	Dose (mL)	Time Given	Time Removed	4 Hr*	24 Hr	48 hr	72 Hr	7 Day	14 Day	Final Weight (kg)
25	377	♂	3.00	0.5	0921.5	1326	Ery +4 Ed + 4	Ery +4 Ed +3	Ery +4 Ed +3	Ery +4 Ed +3	Ery +4 Ed - +3	Ery +4 Ed - +3	3.17
32	392	♂	2.85	0.5	0922.5	1327	Ery +4 Ed +4	Ery +4 Ed +3	Ery +4 Ed +3	Ery +4 Ed +3	Ery +4 Ed - +3	Ery +4 Ed - +3	2.81
33	391	♂	3.16	0.5	0923	1329	Ery +4 Ed +4	Ery +4 Ed +3	Ery +4 Ed +4	Ery +4 Ed +2	Ery +4 Ed - +3	Ery +3 Ed - +3	2.97
70	1733	♀	2.69	0.5	0923.5	1330	Ery +4 Ed +4	Ery +4 Ed +3	Ery +4 Ed +3	Ery +4 Ed +3	Ery +4 Ed - +3	Ery +4 Ed - +2	3.38
69	1737	♀	2.42	0.5	0924	1331	Ery +4 Ed +4	Ery +4 Ed +3	Ery +4 Ed +3	Ery +4 Ed +2	Ery +4 Ed - +3	Ery +4 Ed - +2	2.67
77	1732	♀	2.70	0.5	0925	1332.5	Ery +4 Ed +4	Ery +4 Ed +3	Ery +4 Ed +4	Ery +4 Ed +3	Ery +4 Ed - +3	Ery +4 Ed - +3	2.99

*-denotes contact time of 4 hours with initial observations recorded 30-60 minutes after removal of test material.

Date: 10 Aug 95 **Compound Identification:** DCDMH Oxidizer/Co-solvent Mixture (DCDMH/Chloroform/t-Butyl alcohol) **Physical State**: Liquid

Type of Test: Skin Irritation **Animal Strain/Species:** NZW Rabbit **pH:** N/A

Operators: K. Cameron, J. Manthei, D. Heithamp, D. Bona

Notes and Observations: Skin "draws up" immediately upon dosing. At 7-days, edema still present, although mild. Eschar dry, cracked and peeling. Some scar tissue evident beneath the eschar. Necrotic tissue also evident. Deep subcutaneous tissue damage with blanched areas evident indicating damage to the muscle layer.

TABLE K-5 Irritation Response Data: 4-hour Occluded Exposure to Modified "Red Process Chemistry" Wastestream

Dermal Irritation Testing
(4-hour Occluded)

Animal #	Ear Tag	Sex	Weight (kg)	Dose (mL)	Time Given	Time Removed	4 Hr*	24 Hr	48 hr	72 Hr	7 Day	14 Day	Final Weight (kg)
							OBSERVATION PERIODS						
36	384	♂	2.82	0.5	0917.5	1318	Ery +2 Ed +4	Ery +2 Ed +2	Ery +4 Ed +2	Ery +4 Ed +1	Ery +4 Ed - None	Ery +4 Ed - None	3.00
34	378	♂	2.85	0.5	0918	1319	Ery +1 Ed +4	Ery +2 Ed +2	Ery +4 Ed +2	Ery +4 Ed +1	Ery +4 Ed - None	Ery. +4 Ed - None	3.00
26	385	♂	3.09	0.5	0918.5	1320	Ery +2 Ed +4	Ery +3 Ed +3	Ery +3 Ed +2	Ery +3 Ed +2	Ery +4 Ed - None	Ery +3 Ed - None	3.27
62	1714	♀	2.61	0.5	0919	1321.5	Ery +1 Ed +4	Ery +2 Ed +3	Ery +3 Ed +2	Ery +4 Ed +2	Ery +4 Ed - None	Ery +4 Ed - None	2.88
47	1736	♀	2.38	0.5	0919.5	1323	Ery +1 Ed +4	Ery +2 Ed +3	Ery +2 Ed +2	Ery +2 Ed +1	Ery +4 Ed - None	Ery +4 Ed - None	2.69
48	1740	♀	2.29	0.5	0920	1325	Ery +2 Ed +4	Ery +2 Ed +3	Ery +2 Ed +2	Ery +2 Ed +2	Ery +4 Ed - None	Ery +4 Ed - None	2.48

*-denotes contact time of 4 hours with initial observations recorded 30-60 minutes after removal of test material.

Date: 10 Aug 95

Compound Identification: Modified "Red Process" Wastestream (DCDMH as oxidant)

Physical State: Liquid

Type of Test: Skin Irritation

Animal Strain/Species: NZW Rabbit

pH: N/A

Operators: K. Cameron, J. Manthei, D. Heithamp, D. Bona

Notes and Observations: Skin "draws up" immediately upon dosing.

Blank

APPENDIX L

DERMAL IRRITATION RESPONSE DATA: 4-HR OCCLUDED EXPOSURE TO MODIFIED "CHARCOAL PROCESS" REAGENTS AND WASTESTREAM

TABLES

TABLE L-1 Irritation Response Data: 4-hr Occluded Exposure to Oxidant/Solvent System ("Charcoal Process Chemistry")

Dermal Irritation Testing
(4-hr Occluded Exposure)

OBSERVATION PERIODS

Animal #	Ear Tag	Sex	Weight (kg)	Dose (mL)	Time Given	Time Removed	4 Hr*	24 Hr	48 hr	72 Hr	7 Day	14 Day	Final Weight (kg)
1	375	♂	3.04	0.5	0949.5	1351	Ery +3 Ed +4	Ery +3 Ed +4	Ery +4 Ed +4	Ery +4 Ed +4	Ery +4 Ed +4	Ery None Ed None	3.22
2	410	♂	3.18	0.5	0950	1352	Ery +3 Ed +4	Ery +3 Ed +4	Ery +4 Ed +4	Ery +4 Ed +4	Ery +4 Ed +4	Ery +4 Ed None	3.32
13	406	♂	3.13	0.5	0950.5	1353.5	Ery +3 Ed +4	Er +3 Ed +4	Ery +4 Ed +4	Ery +4 Ed +4	Ery +4 Ed +4	Ery +4 Ed None	2.64
61	1747	♀	2.31	0.5	0951.5	1355	Ery +3 Ed +4	Ery +3 Ed +4	Ery +4 Ed +4	Ery +4 Ed +4	Ery +4 Ed +4	Ery None Ed None	2.71
55	1748	♀	2.45	0.5	0952	1356	Ery +3 Ed +4	Ery +3 Ed +4	Ery +4 Ed +4	Ery +4 Ed +4	Ery +4 Ed +4	Ery None Ed None	2.69
49	1729	♀	2.45	0.5	09525	13557.5	Ery +3 Ed +4	Ery +3 Ed +4	Ery +4 Ed +4	Ery +4 Ed +4	Ery +4 Ed +4	Ery None Ed None	2.72

*-denotes contact time of 4 hours with initial observations recorded 30-60 minutes after removal of test material.

Date: 16 August 95 **Compound Identification:** Oxidizer/Co-Solvent Mixture (DCDMH/chloroform/t-butanol) **Physical State**: Liquid

Type of Test: Dermal Irritation **Animal Strain/Species:** NZW Rabbit **pH:** N/A

Operators: K. Cameron, J. Manthei, D. Heithamp, D. Bona

Notes and Observations:

TABLE L-2 Irritation Response Data: 4-hr Occluded Exposure to Modified "Charcoal Process Chemistry"

Dermal Irritation Testing
(4-hr Occluded Exposure)

OBSERVATION PERIODS

Animal #	Ear Tag	Sex	Weight (kg)	Dose (mL)	Time Given	Time Removed	4 Hr*	24 Hr	48 hr	72 Hr	7 Day	14 Day	Final Weight (kg)
37	407	♂	3.05	0.5	0944	1345	Ery +2 Ed +4	Ery +3 Ed +4	Ery +4 Ed +4	Ery +4 Ed +4	Ery +4 Ed +4	Ery None Ed None	3.30
24	380	♂	2.97	0.5	0944.5	1346	Ery +2 Ed +4	Ery +3 Ed +4	Ery +4 Ed +4	Ery +4 Ed +4	Ery +4 Ed +4	Ery None Ed None	3.19
20	411	♂	3.04	0.5	0945	1347.5	Ery +2 Ed +4	Er +3 Ed +4	Ery +4 Ed +4	Ery +4 Ed +4	Ery +4 Ed +4	Ery None Ed None	3.20
71	1720	♀	2.70	0.5	0946	1348	Ery +2 Ed +4	Ery +2 Ed +4	Ery +4 Ed +4	Ery +4 Ed +4	Ery +4 Ed +4	Ery None Ed None	3.02
65	1744	♀	2.50	0.5	0946.5	1349	Ery +2 Ed +4	Ery +2 Ed +4	Ery +4 Ed +4	Ery +4 Ed +4	Ery +4 Ed +4	Ery None Ed None	2.83
57	1715	♀	2.73	0.5	0947.5	1350	Ery +2 Ed +4	Ery +2 Ed +4	Ery +4 Ed +4	Ery +4 Ed +4	Ery +4 Ed +4	Ery None Ed None	2.96

*-denotes contact time of 4 hours with initial observations recorded 30-60 minutes after removal of test material.

Date: 16 August 95 **Compound Identification:** Charcoal Wastestream Mixture (DCDMH as oxidant) **Physical State:** Liquid

Type of Test: Dermal Irritation **Animal Strain/Species:** NZW Rabbit **pH:** N/A

Operators: K. Cameron, J. Manthei, D. Heithamp, D. Bona

Notes and Observations:

Blank

APPENDIX M

IRRITATION RESPONSE DATA: 4-HR OCCLUDED EXPOSURE TO VESICANT AGENTS (HD, HN, L)

TABLES

TABLE M-1 Irritation Response Data: 4-hour Occluded Exposure To Sulfur Mustard

<u>Dermal Irritation Testing</u>
(4-hour Occluded Exposure)

OBSERVATION PERIODS

Animal #	Ear Tag	Sex	Weight (kg)	Dose (mL)	Time Given	Time Removed	4 Hr*	24 Hr	48 hr	72 Hr	7 Day	14 Day	Final Weight (kg)
17	1187	♂	2.75	0.5	1010	1411	Ery +2 Ed None	Ery +2 Ed +1	Ery +2 Ed +1	Ery +1 Ed None	Ery - None Ed - None	Ery - None Ed - None	3.07
18	1170	♂	2.61	0.5	1010.5	1412	Ery +2 Ed None	Ery +2 Ed +1	Ery +1 Ed None	Ery +1 Ed None	Ery None Ed - None	Ery None Ed - None	2.95
19	099	♀	2.90	0.5	1011.5	1413	Ery +2 Ed None	Ery +2 Ed +1	Ery +1 Ed None	Ery +1 Ed +4	Ery +3 Ed - None	Ery None Ed - None	3.28
20	094	♀	2.94	0.5	1012	1414	Ery +2 Ed None	Ery +2 Ed +1	Ery +2 Ed +1	Ery +4 Ed +4	Ery +4 Ed - +1	Ery None Ed - +1	3.10

*-denotes contact time of 4 hours with initial observations recorded 30-60 minutes after removal of test material.

Date: 21 March 95 **Compound Identification:** HD Control **(109μg/mL in isopropanol)** **Physical State:** Liquid

Type of Test: Skin Irritation **Animal Strain/Species:** NZW Rabbit **pH:** N/A

Operators: K. Cameron, J. Manthei, D. Heithamp, D. Bona

Notes and Observations:

TABLE M-2 Irritation Response Data: 4-hour Occluded Exposure to Sulfur Mustard

Dermal Irritation Testing

(4-hour Occluded Exposure)

OBSERVATION PERIODS

Animal #	Ear Tag	Sex	Weight (kg)	Dose (mL)	Time Given	Time Removed	4 Hr*	24 Hr	48 hr	72 Hr	7 Day	14 Day	Final Weight (kg)
25	167	♂	2.85	0.5	0916	1316	Ery +1 Ed + 4	Ery +3 Ed +4	Ery +4 Ed +4	Ery +4 Ed +4	Ery +4 Ed - +2	Ery - Healed Ed - None	2..96
26	169	♂	2.95	0.5	0916.5	1317	Ery +1 Ed +4	Ery +2 Ed +4	Ery +4 Ed +4	Ery +4 Ed +4	Ery +4 Ed - +2	Ery - Healed Ed - None	3.09
27	178	♂	3.24	0.5	0917	1318	Ery +1 Ed +4	Ery +1 Ed +4	Ery +4 Ed +4	Ery +4 Ed +4	Ery +4 Ed - +3	Ery - Healed Ed - None	3.24
28	1476	♀	3.11	0.5	0917.5	1319.5	Ery +1 Ed +3	Ery +2 Ed +4	Ery +4 Ed +4	Ery +4 Ed +4	Ery +4 Ed - +2	Ery - Healed Ed - None	2.97
29	1473	♀	2.96	0.5	0918.5	1320.5	Ery +1 Ed +3	Ery +1 Ed +4	Ery +4 Ed +4	Ery +4 Ed +4	Ery +4 Ed - +2	Ery - Healed Ed - None	3.05
30	1466	♀	2.91	0.5	0919	1322	Ery +1 Ed +2	Ery +2 Ed +3	Ery +4 Ed +4	Ery +4 Ed +4	Ery +4 Ed - +2	Ery- Healed Ed - None	3.11

*-denotes contact time of 4 hours with initial observations recorded 30-60 minutes after removal of test material.

Date: 6 June 95 **Compound Identification:** HD Control **(50 µg/mL in isopropanol)** **Physical State**: Liquid

Type of Test: Skin Irritation **Animal Strain/Species:** NZW Rabbit **pH:** N/A

Operators: K. Cameron, J. Manthei, D. Heithamp, D. Bona

Notes and Observations:

TABLE M-3 Irritation Response Data: 4-hour Occluded Exposure to Nitrogen Mustard (HN-3)

Dermal Irritation Testing
(4-hour Occluded Exposure)

Animal #	Ear Tag	Sex	Weight (kg)	Dose (mL)	Time Given	Time Removed	OBSERVATION PERIODS 4 Hr*	24 Hr	48 hr	72 Hr	7 Day	14 Day	Final Weight (kg)
31	162	♂	2.70	0.5	0920.5	1323	Ery +1 Ed +1	Ery +2 Ed +3	Ery +2 Ed +4	Ery +3 Ed +4	Ery +4 Ed - +2	Ery - Healed Ed - None	2.67
32	173	♂	3.06	0.5	0921.5	1324.5	Ery +1 Ed +4	Ery +2 Ed +4	Ery +4 Ed +4	Ery +4 Ed +4	Ery +4 Ed - +2	Ery - Healed Ed - None	2.95
33	172	♂	2.94	0.5	0922	1325.5	Ery +1 Ed +2	Ery +3 Ed +4	Ery +3 Ed +4	Ery +3 Ed +4	Ery +3 Ed - +1	Ery - +2 Ed - +1	2.89
34	1464	♀	2.95	0.5	0923	1327	Ery +1 Ed +2	Ery +3 Ed +4	Ery +3 Ed +4	Ery +3 Ed +4	Ery +3 Ed - +1	Ery - Healed Ed - None	2.77
35	1462	♀	3.26	0.5	0923.5	1328	Ery +1 Ed +3	Ery +1 Ed +4	Ery +3 Ed +4	Ery +3 Ed +4	Ery +3 Ed - +1	Ery - Healed Ed - None	3.01
36	1456	♀	2.98	0.5	0924	1329	Ery +1 Ed +3	Ery +1 Ed +3	Ery +3 Ed +4	Ery +3 Ed +4	Ery +3 Ed - +1	Ery- Healed Ed - None	2.89

*-denotes contact time of 4 hours with initial observations recorded 30-60 minutes after removal of test material.

Date: 6 June 95 **Compound Identification:** HN-3 Control **(50 µg/mL in isopropanol)** **Physical State**: Liquid

Type of Test: Skin Irritation **Animal Strain/Species:** NZW Rabbit **pH:** N/A

Operators: K. Cameron, J. Manthei, D. Heithamp, D. Bona

Notes and Observations:

TABLE M-4 Irritation Response Data: 4-hour Occluded Exposure to Lewisite

Dermal Irritation Testing
(4-hour Occluded Exposure)

OBSERVATION PERIODS

Animal #	Ear Tag	Sex	Weight (kg)	Dose (mL)	Time Given	Time Removed	4 Hr*	24 Hr	48 hr	72 Hr	7 Day	14 Day	Final Weight (kg)
37	171	♂	2.81	0.5	0925.5	1330	Ery +1 Ed +4	Ery +3 Ed +4	Ery +4 Ed +4	Ery +4 Ed +4	Ery +4 Ed - +2	Ery - Healed Ed - None	2.76
38	177	♂	2.52	0.5	0926	1331	Ery +1 Ed +3	Ery +3 Ed +4	Ery +4 Ed +4	Ery +4 Ed +4	Ery +4 Ed - +4	Ery - +4 Ed - +4	2.44
39	175	♂	2.86	0.5	0926.5	1332	Ery +2 Ed +2	Ery +3 Ed +4	Ery +4 Ed +4	Ery +4 Ed +4	Ery +4 Ed - +4	Ery - +4 Ed - +4	2.81
40	1460	♀	3.10	0.5	0927.5	1333.5	Ery +1 Ed +3	Ery +3 Ed +4	Ery +4 Ed +4	Ery +4 Ed +4	Ery +4 Ed - +4	Ery +4 Ed +4	2.95
41	1478	♀	3.00	0.5	0928	1334	Ery +1 Ed +3	Ery +4 Ed +4	Ery +4 Ed +4	Ery +4 Ed +4	Ery +4 Ed - +4	Ery - +4 Ed - +4	2.91
42	1475	♀	3.20	0.5	0929	1335	Ery +2 Ed +4	Ery +4 Ed +4	Ery +4 Ed +4	Ery +4 Ed +4	Ery +4 Ed - +4	Ery- +4 Ed - +4	2.99

*-denotes contact time of 4 hours with initial observations recorded 30-60 minutes after removal of test material.

Date: 6 June 95 **Compound Identification:** Lewisite **(50 µg/mL in isopropanol)** **Physical State:** Liquid

Type of Test: Skin Irritation **Animal Strain/Species:** NZW Rabbit **pH:** N/A

Operators: K. Cameron, J. Manthei, D. Heithamp, D. Bona

Notes and Observations:

TABLE M-5 Irritation Response Data: 4-hour Occluded Exposure to Nitrogen Mustard (HN-1 vs HN-3)

Dermal Irritation Testing
(4-hour Occluded Response)

OBSERVATION PERIODS

Animal #	Ear Tag	Sex	Weight (kg)	Dose (mL)	Time Given	Time Removed	4 Hr*	24 Hr	48 hr	72 Hr	7 Day	14 Day	Final Weight (kg)
HN-1													
4	1702	♂	2.36	0.5	1019.5	1420	Ery +3 Ed None	Ery +2 Ed +3	Ery +4 Ed +4	Ery +4 Ed +4	Ery +2 Ed +0	Ery +1 Ed None	2.59
5	1710	♂	2.36	0.5	1020	1421	Ery +2 Ed +3	Ery +2 Ed +3	Ery +4 Ed +4	Ery +4 Ed +4	Ery +2 Ed +0	Ery +2 Ed None	2.64
6	1677	♂	2.38	0.5	1020.5	1422	Ery None Ed None	Er +2 Ed +2	Ery +4 Ed +4	Ery +4 Ed +4	Ery +2 Ed +0	Ery +1 Ed None	2.59
54	1667	♀	2.31	0.5	1021	1423	Ery +2 Ed +3	Ery +2 Ed +3	Ery +3 Ed +4	Ery +4 Ed +4	Ery +2 Ed +0	Ery +1 Ed None	2.49
55	1650	♀	2.27	0.5	1021.5	1424	Ery +1 Ed +2	Ery +1 Ed +2	Ery +3 Ed +4	Ery +4 Ed +4	Ery +1 Ed +0	Ery +1 Ed None	2.47
56	1649	♀	2.34	0.5	1022	1425	Ery +1 Ed +3	Ery +2 Ed +2	Ery +3 Ed +4	Ery +4 Ed +4	Ery +2 Ed +0	Ery +1 Ed None	2.51
HN-3													
7	1676	♂	2.35	0.5	1023.5	1426	Ery +1 Ed +2	Ery +2 Ed +2	Ery +3 Ed +4	Ery +4 Ed +4	Ery +2 Ed +0	Ery +2 Ed None	2.54
8	1694	♂	2.45	0.5	1024	1427	Ery +2 Ed +3	Ery +2 Ed +3	Ery +4 Ed +3	Ery +4 Ed +4	Ery +1 Ed +0	Ery +1 Ed None	2.66
9	1693	♂	2.46	0.5	1025	1428	Ery +1 Ed +3	Ery +3 Ed +3	Ery +3 Ed +4	Ery +4 Ed +4	Ery +4 Ed +0	Ery +4 Ed None	2.61
58	1674	♀	2.44	0.5	1025.5	1429	Ery +1 Ed +3	Ery +3 Ed +3	Ery +4 Ed +3	Ery +4 Ed +4	Ery +2 Ed +0	Ery +1 Ed None	2.59
59	1664	♀	2.36	0.5	1026.5	1430	Ery +2 Ed +3	Ery +2 Ed +3	Ery +4 Ed +4	Ery +4 Ed +4	Ery +3 Ed +0	Ery +3 Ed None	2.60
60	1660	♀	2.39	0.5	1027	1431	Ery +1 Ed +3	Ery +2 Ed +3	Ery +3 Ed +4	Ery +4 Ed +4	Ery +1 Ed +0	Ery +1 Ed None	2.54

*-denotes contact time of 4 hours with initial observations recorded 30-60 minutes after removal of test material.

Date: 19 July 95 **Compound Identification:** HN-1/HN-3 Comparison **(50 µg/mL)** **Physical State:** Liquid
Type of Test: Dermal Irritation **Animal Strain/Species:** NZW Rabbit **pH:** N/A
Operators: Cameron, Manthei, Heitkamp, Bona **Notes and Observations:**

APPENDIX N

IRRITATION RESPONSE DATA: 24-HR OCCLUDED EXPOSURE TO "BLUE PROCESS" OXIDANT/SOLVENT SYSTEMS AND WASTESTREAMS

TABLES

[a]- Refer to page 40 (footnote 14) for explanation

TABLE N-1 Irritation Exposure Data: 4-hour Occluded Exposure to Brom55P/Sulfolane Mixture

Dermal Irritation Testing
(4-hour Occluded Exposure)

OBSERVATION PERIODS

Animal #	Ear Tag	Sex	Weight (kg)	Dose (mL/kg)	Time Given	Time Removed	4 Hr	24 Hr	48 Hr	72 hr	7 Day	14 Day	Final Weight (kg)
9	1198	♂	2.65	0.5	0952.5	1354	Ery +1 Ed none	Ery +2 Ed +1	Ery +2 Ed +1	Ery +2 Ery +1	Ery +2 Ed +1	Ery +2 Ed +1	2.86
10	1162	♂	2.92	0.5	0954	1356	Ery +1 Ed none	Ery +2 Ed none	Ery +2 Ed +1	Ery none Ed none	Ery none Ed none	Ery none Ed none	3.29
11	073	♀	2.83	0.5	0954.5	1357	Ery +1 Ed none	Er +3 Ed +3	Ery +4 Ed +4	Ery +4 Ed +4	Ery +4 Ed none	Ery +4 Ed +1	3.05
12	059	♀	3.00	0.5	0955	1359	Ery +1 Ed none	Ery +3 Ed +3	Ery +4 Ed +4	Ery +4 Ed +4	Ery +4 Ed +4	Ery +4 Ed +1	3.41

Date: 21 March 95

Compound Identification: Oxidizer/Solvents (Brom55P/sulfolane mixture)

Physical State: Liquid

Type of Test: Skin Irritation

Animal Strain/Species: NZW Rabbit

pH: N/A

Operators: K. Cameron, J. Manthei, D. Heithamp, D. Bona

Notes and Observations:

TABLE N-2 Irritation Response Data: 24-hour Occluded Exposure to DCDMH/Chloroform/t-Butanol Mixture

Dermal Irritation Testing
(24-hr Occluded Exposure

Animal #	Ear Tag	Sex	Weight (kg)	Dose (mL)	Total Dose	Time Given	Time Removed	24 Hr	48 hr	72 Hr	7 Day	14 Day	Final Weight (kg)
								OBSERVATION PERIODS					
1	1191	♂	2.96	1.0	2.96	0954.5	0955	Ery +2 Ed +4	Ery +4 Ed +4	Ery +4 Ed +4	Ery +4 Ed +4	Ery +4 Ed +4	3.06
2	1195	♂	3.06	1.0	3.06	0955.5	0956	Ery +2 Ed +4	Ery +4 Ed +4	Ery +4 Ed +4	Ery +4 Ed +4	Ery +4 Ed +4	3.20
3	1174	♂	3.28	1.0	3.28	0956.5	0958	Er +2 Ed +4	Ery +4 Ed +4	Ery +4 Ed +4	Ery +4 Ed +4	Ery +4 Ed +4	3.42
4	1179	♂	3.13	1.0	3.13	0957	0959	Ery +4 Ed +4	Ery +4 Ed +4	Ery +4 Ed +4	Ery +4 Ed +4	Ery +4 Ed +4	3.18
5	1298	♂	2.51	1.0	2.51	0958	1000	Ery +4 Ed +4	Ery +4 Ed +4	Ery +4 Ed +4	Ery +4 Ed +4	Ery +4 Ed +4	2.66
6	067	♀	3.00	1.0	3.00	0959	1002	Ery +4 Ed +4	Ery +4 Ed +4	Ery +4 Ed +4	Ery +4 Ed +4	Ery +4 Ed +4	3.04
7	079	♀	2.99	1.0	2.99	1000	1003	Ery +4 Ed +4	Ery +4 Ed +4	Ery +4 Ed +4	Ery +4 Ed +4	Ery +4 Ed +4	2.97
8	064	♀	3.03	1.0	3.03	1001	1004	Ery +4 Ed +4	Ery +4 Ed +4	Ery +4 Ed +4	Ery +4 Ed +4	Ery +4 Ed +4	3.21
9	075	♀	3.15	1.0	3.15	1002	1006	Ery +4 Ed +4	Ery +4 Ed +4	Ery +4 Ed +4	Ery +4 Ed +4	Ery +4 Ed +4	3.10
10	093	♀	3.38	1.0	3.38	1003	1008	Ery +4 Ed +4	Ery +4 Ed +4	Ery +4 Ed +4	Ery +4 Ed +4	Ery +4 Ed +4	3.40

Date: 12 April 95 **Compound Identification:** Oxider/Co-solvent Mixture (DCDMH/chloroform/t-butanol) **Physical State:** Liquid **pH:** N/A

Type of Test: Skin Irritation **Animal Strain/Species:** NZW Rabbit **Operators:** K. Cameron, J. Manthei, D. Heithamp, D. Bona

TABLE N-3 Irritation Exposure Data: 24-hour Occluded Exposure to Initial "Blue Process Chemistry" Wastestream

Dermal Irritation Testing
(24-hour Occluded Exposure)

Animal #	Ear Tag	Sex	Weight (kg)	Dose (mL/kg)	Time Given	Time Removed	OBSERVATION PERIODS 2 4 Hr	48 Hr	72 hr	7 Day	14 Day	Final Weight (kg)
1	899	♀	3.40	1.0	0953	0956	Ery +4 Ed +4	Ery +4 Ed +4	Ery +4 Ery +4	Ery +4 Ed +4	Ery +4 Ed +4	3.26
2	805	♀	3.15	1.0	0955	0958	Ery +4 Ed +4	Ery +4 Ed +4	Ery +4 Ed +4	Ery +4 Ed +4	Ery +4 Ed +4	3.05
3	892	♀	3.65	1.0	0932	0934	Ery +4 Ed +4	Er +4 Ed +4	Ery +4 Ed +4	Ery +4 Ed +4	Not Observed	3.41 (7-days)
4	804	♀	3.50	1.0	0937	0940	Ery +4 Ed +4	Ery +4 Ed +4	Ery +4 Ed +4	Ery +4 Ed +4	Not Observed	3.45 (7-days)

Date: 24 and 31 Jan 95

Compound Identification: Wastestream Mixture (Brom55P/sulfolane/HD)

Physical State: Liquid

Type of Test: Skin Irritation

Animal Strain/Species: NZW Rabbit

pH: N/A

Operators: K. Cameron, J. Manthei, D. Heithamp, D. Bona

Notes and Observations:

TABLE N-4 Irritation Response Data: 24-hr Occluded Exposure to Modified "Blue Process Chemistry" Wastestream

Dermal Irritation Testing
(24-hr Occluded Exposure)

OBSERVATION PERIODS

Animal #	Ear Tag	Sex	Weight (kg)	Dose (mL)	Total Dose	Time Given	Time Removed	24 Hr	48 hr	72 Hr	7 Day	14 Day	Final Weight (kg)
11	1290	♂	2.49	1.0	2.49	1005	1005	Ery +1 Ed +4	Ery +3 Ed +4	Ery +3 Ed +4	Ery +4 Ed +4	Ery +4 Ed None	2.69
12	1311	♂	2.46	1.0	2.46	1006	1007	Ery +1 Ed +4	Ery +3 Ed +4	Ery +3 Ed +4	Ery +4 Ed +4	Ery +4 Ed None	2.60
13	1192	♂	3.18	1.0	3.18	1007	1009	Er +4 Ed +4	Ery +4 Ed +4	Ery +4 Ed +4	Ery +4 Ed +4	Ery +4 Ed None	3.24
14	1200	♂	3.22	1.0	3.22	1008	1010	Ery +4 Ed +4	Ery +4 Ed +4	Ery +4 Ed +4	Ery +4 Ed +4	Ery +4 Ed None	3.16
15	1184	♂	3.21	1.0	3.21	1009	1011	Ery +4 Ed +4	Ery +4 Ed +4	Ery +4 Ed +4	Ery +4 Ed +4	Ery +4 Ed None	3.14
16	081	♀	3.33	1.0	3.33	1009.5	1012	Ery +4 Ed +4	Ery +4 Ed +4	Ery +4 Ed +4	Ery +4 Ed +4	Ery +4 Ed None	3.00
17	082	♀	3.16	1.0	3.16	1010.5	1014	Ery +4 Ed +4	Ery +4 Ed +4	Ery +4 Ed +4	Ery +4 Ed +4	Ery +4 Ed None	3.19
18	086	♀	3.10	1.0	3.10	1011.5	1015	Ery +3 Ed +4	Ery +3 Ed +4	Ery +3 Ed +4	Ery +4 Ed +4	Ery +4 Ed None	3.07
19	096	♀	3.24	1.0	3.24	1012.5	1017	Ery +4 Ed +4	Ery +4 Ed +4	Ery +4 Ed +4	Ery +4 Ed +4	Not Observed (Sacrificed)	3.00
20	066	♀	3.03	1.0	3.03	1013	1019	Ery +4 Ed +4	Ery +4 Ed +4	Ery +4 Ed +4	Ery +4 Ed +4	Ery +4 Ed None	3.01

Date: 12 April 95 **Compound Identification:** Modified Blue Process Wastestream (DCDMH as oxidant) **Physical State:** Liquid **pH:** N/A

Type of Test: Skin Irritation **Animal Strain/Species:** NZW Rabbit **Operators:** K. Cameron, J. Manthei, D. Heithamp, D. Bona

Blank

APPENDIX O

IRRITATION RESPONSE DATA: 24-HR OCCLUDED EXPOSURE TO "RED PROCESS" OXIDANT/SOLVENT SYSTEMS AND WASTESTREAMS

TABLE

TABLE O-1 Irritation Response Data: 24-hour Occluded Exposure to m-cpba/$CHCl_3$/t-BuOH

Dermal Irritation Testing

(24-hr Occluded Exposure

OBSERVATION PERIODS

Animal #	Ear Tag	Sex	Weight (kg)	Dose (mL/kg)	Total Dose	Time Given	Time Removed	24 Hr	48 hr	72 Hr	7 Day	14 Day		Final Weight (kg)
1	1193	♂	2.95	1.0	2.95	0934	0935	Ery None Ed +4	Ery +4 Ed +4	Ery +4 Ed +4	Ery +4 Ed +4	Ery None Ed None		3.04
2	1177	♂	2.87	1.0	2.87	0935	0937	Ery None Ed +4	Ery +4 Ed +4	Ery +4 Ed +4	Ery +4 Ed +4	Ery None Ed None		3.12
3	100	♀	3.00	1.0	3.00	0936	0939	Ery None Ed +4	Ery +4 Ed +4	Ery +4 Ed +4	Ery +4 Ed +4	Ery None Ed None		3.14
4	083	♀	3.04	1.0	3.04	0938	0942	Ery None Ed +4	Ery +4 Ed +4	Ery +4 Ed +4	Ery +4 Ed +4	Ery Healing Ed +4		3.18

Date: 29 March 95 **Compound Identification:** Oxidizer/Solvent (m-CPBA/chloroform/t-butanol) **Physical State:** Liquid **pH:** N/A

Type of Test: Skin Irritation **Animal Strain/Species:** NZW Rabbit **Operators:** K. Cameron, J. Manthei, D. Heithamp, D. Bona

TABLE O-2 Irritation Response Data: 24-hour Occluded Exposure to Initial "Red Process Chemistry" Wastestream

Dermal Irritation Testing
(24-hr Occluded Exposure)

Animal #	Ear Tag	Sex	Weight (kg)	Dose (mL/kg)	Total Dose	Time Given	Time Removed	OBSERVATION PERIODS 24 Hr	48 hr	72 Hr	7 Day	14 Day	Final Weight (kg)
5	1181	♂	2.80	1.0	2.80	0941	0944	Died<24hrs					2.80
6	1172	♂	3.05	1.0	3.05	0943	0947	Ery +4 Ed +4	Ery +4 Ed +4	Ery +4 Ed +4	Died >96 hrs <5 days		3.12
7	089	♀	3.15	1.0	3.15	0945	0950	Ery +4 Ed +4	Ery +4 Ed +4	Ery +4 Ed +4	Died 6 days		
8	095	♀	2.93	1.0	2.93	0946	0946	Ery +4 Ed +4	Ery +4 Ed +4	Ery +4 Ed +4	Ery +4 Ed +4	Ery +4 Ed +4	2.83
9	1186	♂	2.82	1.0	2.82	1011	1012	Ery +4 Ed +4	Ery +4 Ed +4	Ery +4 Ed +4	Ery +4 Ed +4	Ery +4 Ed +4	2.71
10	1168	♂	3.06	1.0	3.06	1012.5	1016	Ery +4 Ed +4	Ery +4 Ed +4	Ery +4 Ed +4	Ery +4 Ed +4	Ery +4 Ed +4	2.98
11	1161	♂	2.87	1.0	2.87	1014	1018	Ery +4 Ed +4	Ery +4 Ed +4	Died 72 hrs			2.61
12	070	♀	2.80	1.0	2.80	1015.5	1021	Ery +4 Ed +4	Ery +4 Ed +4	Ery +4 Ed +4	Ery +4 Ed +4	Ery +4 Ed +4	2.75
13	098	♀	3.03	1.0	3.03	1017	1023	Ery +4 Ed +4	Ery +4 Ed +4	Ery +4 Ed +4	Ery +4 Ed +4	Ery +4 Ed +4	2.90
14	061	♀	2.76	1.0	2.76	1018.5	1025	Ery +4 Ed +4	Ery +4 Ed +4	Ery +4 Ed +4	Ery +4 Ed +4	Ery +4 Ed +4	2.60
15	1297	♂	2.54	1.0	2.54	0937.5	0938	Ery +4 Ed +4	Ery +4 Ed +4	Ery +4 Ed +4	Ery +4 Ed +4	Ery +4 Ed +4	2.37
16	1288	♂	2.37	1.0	2.37	0939	0940	Ery +4 Ed +4	Ery +4 Ed +4	Ery +4 Ed +4	Ery +4 Ed +4	Ery +4 Ed +4	2.41
17	065	♀	3.04	1.0	3.04	0940.5	0942	Ery +4 Ed +4	Ery +4 Ed +4	Ery +4 Ed +4	Ery +4 Ed +4	Ery +4 Ed +4	3.04
18	078	♀	2.97	1.0	2.97	0942	0942	Ery +4 Ed +4	Ery +4 Ed +4	Ery +4 Ed +4	Ery +4 Ed +4	Ery +4 Ed +4	2.97

Date: 29 March 95 **Compound Identification:** Initial Red Process Wastestream (m-CPBA as oxidizer) **Physical State:** Liquid **pH:** N/A

Type of Test: Skin Irritation **Animal Strain/Species:** NZW Rabbit **Operators:** K. Cameron, J. Manthei, D. Heithamp, D. Bona

TABLE O-3 Irritation Response Data: 24-hour Occluded Exposure to Modified "Red Process Chemistry" Wastestream

Dermal Irritation Testing

(24-hr Occluded Exposure)

Animal #	Ear Tag	Sex	Weight (kg)	Dose (mL/kg)	Total Dose	Time Given	Time Removed	OBSERVATION PERIODS 24 Hr	48 hr	72 Hr	7 Day	14 Day	Final Weight (kg)
1	1188	♂	3.24	1.0	3.24	0947	0945	Ery +4 Ed +4	Ery +4 Ed +4	Ery +4 Ed +4	Ery +4 Ed +2	Ery +4 Ed +None	3.22
2	1190	♂	3.03	1.0	3.03	0953	0955	Ery +4 Ed +4	Ery +4 Ed +4	Ery +4 Ed +4	Ery +4 Ed +2	Ery +4 Ed +1	3.07
3	1197	♂	2.96	1.0	2.96	0954	0956	Ery +4 Ed +4	Ery +4 Ed +4	Ery +4 Ed +4	Ery +4 Ed +2	Ery +4 Ed +None	3.03
4	1176	♂	3.32	1.0	3.32	0955	0957	Ery +4 Ed +4	Ery +4 Ed +4	Ery +4 Ed +4	Ery +4 Ed +2	Ery +4 Ed +None	3.39
5	1169	♂	3.10	1.0	3.10	0955.5	0958	Ery +4 Ed +4	Ery +4 Ed +4	Ery +4 Ed +4	Ery +4 Ed +2	Ery +4 Ed +1	3.02
6	072	♀	3.50	1.0	3.50	0957.5	0959.5	Ery +4 Ed +4	Ery +4 Ed +4	Ery +4 Ed +4	Ery +4 Ed +3	Ery +4 Ed +1	3.44
7	091	♀	3.26	1.0	3.26	0958	1000.5	Ery +4 Ed +4	Ery +4 Ed +4	Ery +4 Ed +4	Ery +4 Ed +2	Ery +4 Ed +None	3.24
8	085	♀	3.16	1.0	3.16	0958.5	1006	Ery +4 Ed +4	Ery +4 Ed +4	Ery +4 Ed +4	Ery +4 Ed +2	Ery +4 Ed +None	3.31
9	069	♀	3.04	1.0	3.04	0959.5	1008	Ery +4 Ed +4	Ery +4 Ed +4	Ery +4 Ed +4	Ery +4 Ed +3	Ery +4 Ed +None	3.08
10	092	♀	3.38	1.0	3.38	1000.5	1009.5	Ery +4 Ed +4	Ery +4 Ed +4	Ery +4 Ed +4	Ery +4 Ed +3	Ery +4 Ed +None	3.40

Date: 18 April 95 **Compound Identification:** Red Process Wastestream (DCDMH/chloroform/t-butanol) **Physical State:** Liquid **pH:** N/A

Type of Test: Skin Irritation **Animal Strain/Species:** NZW Rabbit **Operators:** K. Cameron, J. Manthei, D. Heithamp, D. Bona

APPENDIX P

IRRITATION RESPONSE DATA: 24-HR OCCLUDED EXPOSURE TO “CHARCOAL PROCESS” OXIDANT/SOLVENT SYSTEM AND WASTESTREAM

TABLES

TABLE P-1 Irritation Exposure Data: 24-hour Occluded Exposure to Charcoal Process Oxidant/Solvent System

Dermal Irritation Testing
(24-hour Occluded Exposure)

Animal #	Ear Tag	Sex	Weight (kg)	Dose (mL)	Time Given	Time Removed	OBSERVATION PERIODS: 2 4 Hr	48 Hr	72 hr	7 Day	14 Day	Final Weight (kg)
29	403	♂	3.13	1.0	0955.5	0956	Ery +4 Ed +4	Ery +4 Ed +4	Ery +4 Ery +4	Ery +4 Ed +4	Ery Healing Ed None	3.17
31	398	♂	3.18	1.0	0956.5	0958	Ery +4 Ed +4	Ery +4 Ed +4	Ery +4 Ed +4	Ery +4 Ed +4	Ery Healing Ed None	3.13
30	393	♂	3.27	1.0	0958.5	1000	Ery +4 Ed +4	Er +4 Ed +4	Ery +4 Ed +4	Ery +4 Ed +4	Ery Healing Ed None	3.22
38	376	♂	3.08	1.0	1000	1001	Ery +4 Ed +4	Ery +4 Ed +4	Ery +4 Ed +4	Ery +4 Ed +4	Ery Healing Ed +1	3.16
28	404	♂	2.92	1.0	1001	1002.5	Ery +4 Ed +4	Ery +4 Ed +4	Ery +4 Ed +4	Ery +4 Ed +4	Ery +4 Ed None	3.04
51	1723	♀	2.78	1.0	1002.5	1003.5	Ery +4 Ed +4	Ery +4 Ed +4	Ery +4 Ed +4	Ery +4 Ed +4	Ery +4 Ed None	2.72
74	1726	♀	2.70	1.0	1004	1004.5	Ery +4 Ed +4	Ery +4 Ed +4	Ery +4 Ed +4	Ery +4 Ed +4	Ery Healing Ed None	2.93
53	1746	♀	2.30	1.0	1005.5	1006	Ery +4 Ed +4	Ery +4 Ed +4	Ery +4 Ed +4	Ery +4 Ed +4	Ery +4 Ed None	2.45
60	1727	♀	2.44	1.0	1006.5	1008	Ery +4 Ed +4	Ery +4 Ed +4	Ery +4 Ed +4	Ery +4 Ed +4	Ery Healing Ed None	2.66
52	1749	♀	2.38	1.0	1008	1009.5	Ery +4 Ed +4	Ery +4 Ed +4	Ery +4 Ed +4	Ery +4 Ed +4	Ery +4 Ed None	2.68

Date: 16 August 95

Compound Identification: Oxidizer/Solvents (DCDMH/chloroform/t-butanol)

Physical State: Liquid

Type of Test: Skin Irritation

Animal Strain/Species: NZW Rabbit

pH: N/A

Operators: K. Cameron, J. Manthei, D. Heithamp, D. Bona

Notes and Observations:

TABLE P-2 Irritation Response Data: 24-hour Occluded Exposure to Charcoal Process Wastestream

Dermal Irritation Testing
(24-hr Occluded Exposure)

OBSERVATION PERIODS

Animal #	Ear Tag	Sex	Weight (kg)	Dose (mL)	Time Given	Time Removed	2 4 Hr	48 Hr	72 hr	7 Day	14 Day	Final Weight (kg)
1	1165	♂	3.46	1.0	0955.5	0932	Ery +3 Ed +4	Ery +4 Ed +4	Ery +4 Ery +4	Ery +4 Ed +4	Ery +4 Ed None	3.72
2	1159	♂	3.46	1.0	0932	0933	Ery +3 Ed +4	Ery +4 Ed +4	Ery +4 Ed +4	Ery +4 Ed +4	Ery +4 Ed None	3.57
3	168	♂	2.46	1.0	0933	0934	Ery +4 Ed +4	Er +4 Ed +4	Ery +4 Ed +4	Ery +4 Ed +4	Ery +4 Ed None	2.81
4	163	♂	2.46	1.0	0933.5	0935	Ery +2 Ed +4	Ery +3 Ed +4	Ery +3 Ed +4	Ery +4 Ed +4	Ery +4 Ed None	2.80
5	174	♂	2.70	1.0	0934.5	0936	Ery +4 Ed +4	Ery +4 Ed +4	Ery +4 Ed +4	Ery +4 Ed +4	Ery +4 Ed None	2.94
6	080	♀	3.64	1.0	0935.5	0937	Ery +2 Ed +4	Ery +4 Ed +4	Ery +4 Ed +4	Ery +4 Ed +4	Ery +4 Ed None	3.71
7	068	♀	3.39	1.0	0936.5	0938	Ery +3 Ed +4	Ery +4 Ed +4	Ery +4 Ed +4	Ery +4 Ed +4	Ery +4 Ed None	3.52
8	1465	♀	2.61	1.0	0937	0939	Ery +3 Ed +4	Ery +4 Ed +4	Ery +4 Ed +4	Ery +4 Ed +4	Ery +4 Ed None	2.79
9	1474	♀	2.32	1.0	0938	0940	Ery +2 Ed +4	Ery +4 Ed +4	Ery +4 Ed +4	Ery +4 Ed +4	Ery +4 Ed None	2.64
10	1472	♀	2.61	1.0	0939	0941	Ery +3 Ed +4	Ery +4 Ed +4	Ery +4 Ed +4	Ery +4 Ed +4	Ery +4 Ed None	2.95

Date: 16 May 95

Compound Identification: Charcoal Process Wastestream (DCDMH as oxidant)

Physical State: Liquid

Type of Test: Skin Irritation

Animal Strain/Species: NZW Rabbit

pH: N/A

Operators; K. Cameron, J. Manthei, D. Heithamp, D. Bona

Notes and Observations: